Synthesis and biological activity of mimics of D-myo-inositol 1,4,5-trisphosphate and adenophostin A

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Synthesis and Biological Activity of mimics of D-*myo*-Inositol 1,4,5-Trisphosphate and adenophostin A.

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Abstract

The synthesis of adenophostin A, a recently discovered exceptionally potent agonist at the 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] receptor, and a series of adenophostin A analogues is described. Structure-activity correlations for Ca^{2+} release at the hepatocyte Ins(1,4,5)P_3 receptor are described.

α–And β-*C*-(hydroxymethyl)-1-deoxy-D-glucopyranoside 3,4, 1'-trisphosphates were designed and synthesised as monosaccharide analogues based on an originally described adenophostin A mimic (2-hydroxyethyl-α-D-glucopyranoside-2,3',4'-trisphosphate), but with a shorter and less flexible side chain. The α-trisphosphate was similar in potency for Ca^{2+} release as Ins(1,4,5)P_3 while the β-trisphosphate was considerably weaker. Simplification of the adenophostin A structure led to the design and synthesis of [(3S,4R)-3-hydroxytetrahydrofuran-4-yl] α-D-xylopyranoside 3,3',4-trisphosphate (xylofuranophostin), a disaccharide-derived analogue in which the adenine and both hydroxymethyl moieties have been deleted. In order to explore the effect of stereochemical variations on the biological activity of xylofuranophostin, and in particular the positioning of the non-vicinal phosphate group for potent activity, the synthesis of three of its diastereoisomers was undertaken. Further simplification would inevitably result in a substantial decrease in potency, and xylofuranophostin is therefore likely to represent the simplest possible structure for potent Ca^{2+}-releasing activity in this type of carbohydrate-based analogue.

To enable the synthesis of adenophostin A and base-modified adenophostin A analogues a disaccharide intermediate, 1,2,3',4'-tetra-tri-*O*-acetyl-2',5,6'-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose was synthesised. Vorbrüggen condensation of this intermediate with 6-chloropurine/2,6-dichlorpurine led to the synthesis of a series of adenophostin A analogues elaborated at N-6 and C-2. Biological evaluation of these compounds has shown N6-methyl adenophostin to be amongst the first totally synthetic compounds to approach the activity of adenophostin A. By the introduction of different sized molecules this study has revealed evidence of a possible unoccupied and new receptor binding pocket for the Ins(1,4,5)P_3R in complex with adenophostin A. These results further demonstrate that a base-modification approach represents a powerful strategy to develop high potency ligands.

Publications

Rosenberg, H. J., Riley, A. M., Correa, V., Taylor, C. W., & Potter, B. V. L. (2000) *C*-glycoside based mimics of D-*myo*-inositol 1,4,5-trisphosphate. *Carbohydr. Res.*, **329,** 7-16.

Rosenberg, H. J., Riley, A. M., Marwood, R. D., Correa, V., Taylor, C. W., & Potter, B. V. L. (2001) Xylopyranoside-based agonists of D-*myo*-inositol 1,4,5- trisphosphate receptors: synthesis and effect of stereochemistry on biological activity. *Carbohydr. Res.*, **332,** 53-66.

Rosenberg, H. J., Riley, A. M., Bottril, F., Nerou E. P., Taylor, C. W., & Potter, B. V. L., Purine-modified mimics of adenophostin A: Synthesis and Ca^{2+} mobilising activity. *J. Med. Chem* (manuscript in preparation).

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For pharmacological evaluation of the analogues presented here, I would like to thank our collaborators at the University of Cambridge, Department of Pharmacology, Tennis Court Road, Cambridge.

Dedication

To all my family, with love.

Abbreviations

$[\alpha]_D$	specific rotation at 589 nm
°C	degrees Celsius
Ac	acetyl
AdA	adenophostin A
All	allyl
APB	aminoethoxydiphenylborate
Ar	aryl
ATP	adenosine 5'-triphosphate
9-BBN	9-borabicyclo[3.3.1]nonane
BDA	butane diacetal
Bn	benzyl
bp	boiling point
br	broad (spectral)
BSA	bis(trimethylsilyl)acetamide
Bz	benzoyl
BZDC	*p*-benzoyldihydrocinnamoyl
cADPR	cyclic adenosine 5'-diphosphate ribose
CAM	calmodulin
CDP	cytidine 5'-diphosphate
cGMP	guanosine 3',5'-cyclic phosphate
CICR	Ca^{2+} induced Ca^{2+} release
CIF	Ca^{2+} influx factor
CLM	cytosol-like medium
COSY	correlated spectroscopy
CRAC	Ca^{2+} release activated Ca^{2+} channels
CSA	camphorsulfonic acid
CTP	cytidine 5'-triphosphate
d	doublet (spectral)
DAG	1,2-diacylgylcerol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	doublet of doublets (spectral)
ddd	doublet of doublet of doublets (spectral)
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarisation transfer
DMAP	*N,N*-4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulphoxide
DMTr	dimethoxytrityl

EC_{50}	half maximal effective agonist concentration
ENTH	epsin amino-terminal homology
ER	endoplasmic reticulum
FAB	fast atom bombardment
FERM	four-point-one-ezrin-radixin-moesin
FYVE	Fab1-YOTP-Vac1-EEA1
GDP	guanosine 5'-diphosphate
Glc(2',3,4)P_3	(2-hydroxyethyl)-α-D-glucopyranoside-2',3,4-trisphosphate
GTP	guanosine 5'-triphosphate
h	Hill coefficient
h	hour
HPLC	high-performance liquid chromatography
Hz	Hertz
I_{CRAC}	calcium-release activated calcium current
Ins(1,4,5)P_3	1D-*myo*-inositol 1,4,5-trisphosphate
Ins(1,4,5)P_3R	1D-*myo*-inositol 1,4,5-trisphosphate receptor
IR	infra-red
J	coupling constant (in NMR)
Kd	equilibrium dissociation constant
KDa	kilodalton
L	litre(s)
lit	literature (reference)
m	milli, multiplet (spectral)
M	moles per litre
m/z	mass to charge ratio (mass spectrometry)
*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MHz	megaHertz
min	minute(s)
mM	millimoles per litre
MOE	molecular operating environment
mol	mole(s)
mp	melting point
MS	mass spectrometry
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PCA	principle component analysis
PH	pleckstrin homology
PI	phosphatidylinositol
PIP	phosphatidylinositol phosphate

Piv	pivaloyl
PLC	phospholipase C
PLD	polymer linked dimers
PMB	*p*-methoxybenzyl
ppm	parts per million (in NMR)
PtdA	phosphatidic acid
PtdIns	phosphatidylinositol
PtdIns(4,5)P_2	phosphatidylinositol 4,5-bisphosphate
PtdOH	phosphatidic acid
*p*TSA	*p*-toluenesulphonic acid
PX	phox
q	quartet (spectral)
QSAR	quantitative structure activity relationship
R_f	retention factor (in chromatography)
rt	room temperature
RyR	ryanodine receptors
s	singlet (spectral)
SAR	structure activity relationship
SERCA	sarco endoplasmic reticulum Ca^{2+} ATPase
SOC	store operated channel
t	triplet (spectral)
TEAB	triethylammonium bicarbonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TMSOTf	trimethylsilylmethyl trifluoromethanesulfonate
UV	ultra-violet
VOC	voltage operated channel
δ	chemical shift
μ	micro

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Chapter one

Introduction

Ca^{2+} and $Ins(1,4,5)P_3$

Chapter 1

Introduction Part a: Ca^{2+} and $Ins(1,4,5)P_3$

1.1 Intracellular Calcium Signaling

Intracellular calcium plays a crucial role in the control of many cellular processes as diverse as cell proliferation, muscle contraction, secretion, metabolism and neuronal signaling[1]. In order to accomplish such varying feats the concentration of intracellular Ca^{2+} must be regulated.

When the Ca^{2+} concentration is low (10–100 nM) the cells are at rest but when raised to 500–1000 nM the cells are activated to perform their various functions. The concentration is finely regulated by various mechanisms related to physiological function.

One mechanism is the influx of Ca^{2+} via Ca^{2+} channels, for example voltage-operated channels (VOCs), across the cell membrane. This mechanism takes advantage of the fact that there is a much larger concentration of Ca^{2+} outside the cell than inside therefore producing a large electrochemical gradient favouring Ca^{2+} entry. Another mechanism is the release of Ca^{2+} from intracellular Ca^{2+} stores via intracellular Ca^{2+} release channels. Release from intracellular Ca^{2+} stores is accomplished by small molecules, known as second messengers. These include D-*myo*-inositol 1,4,5-trisphosphate [$Ins(1,4,5)P_3$, figure 1.1], cyclic adenosine diphosphate ribose (cADPR, figure 1.2) and nicotinic acid adenine dinucleotide phosphate (NAADP, figure 1.3)[2]. Ca^{2+} release from $Ins(1,4,5)P_3$ receptor [$Ins(1,4,5)P_3R$] is induced by the second messenger $Ins(1,4,5)P_3$, while RyR is regulated by cADPR[3;4]. Both receptors share many similarities and are modulated by cytoplasmic Ca^{2+} concentrations, calmodulin and phosphorylation. The receptor for NAADP and the Ca^{2+} channel involved have not yet been discovered. However, binding studies using [^{32}P]NAADP revealed a single class of high affinity binding sites different from the RyR and $Ins(1,4,5)P_3R$.

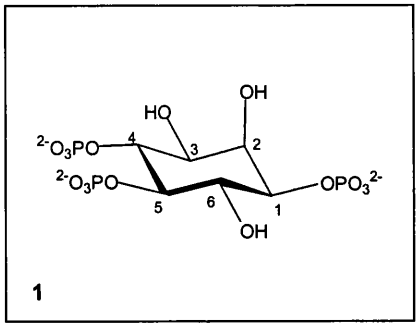


Figure 1.1: $Ins(1,4,5)P_3$

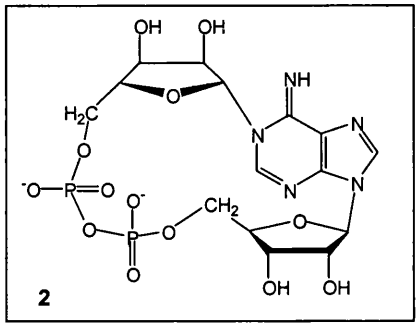


Figure 1.2: Cyclic ADP-ribose

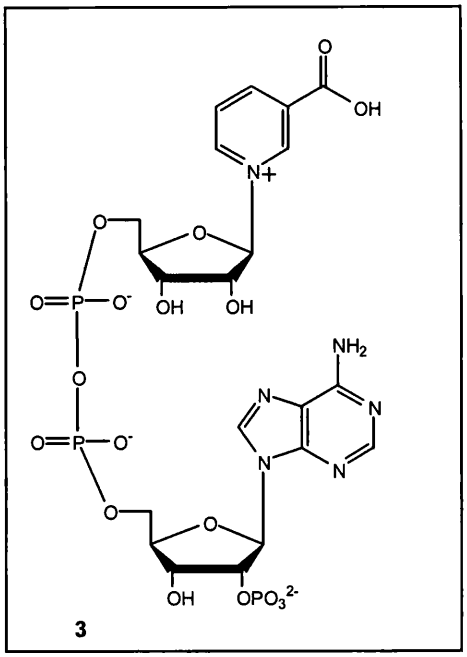


Figure 1.3: NAADP

The rest of the thesis will be concerned with $Ins(1,4,5)P_3$, adenophostin (introduced in section 1.12) and their analogues.

1.2 Phosphoinositides and the origin of $Ins(1,4,5)P_3$

When the receptor on the surface of the cell membrane is activated it triggers phospholipase C (PLC) enzymes[5] to break down the membrane-bound phosphatidylinositol 4,5-bisphosphate [$PtdIns(4,5)P_2$] into diacyl glycerol (DAG) and $Ins(1,4,5)P_3$. The latter diffuses into the cytoplasm, binds to its receptor and releases stored Ca^{2+}. Some PLC enzymes are regulated by G-nucleotide-binding proteins and others by receptor tyrosine kinases.

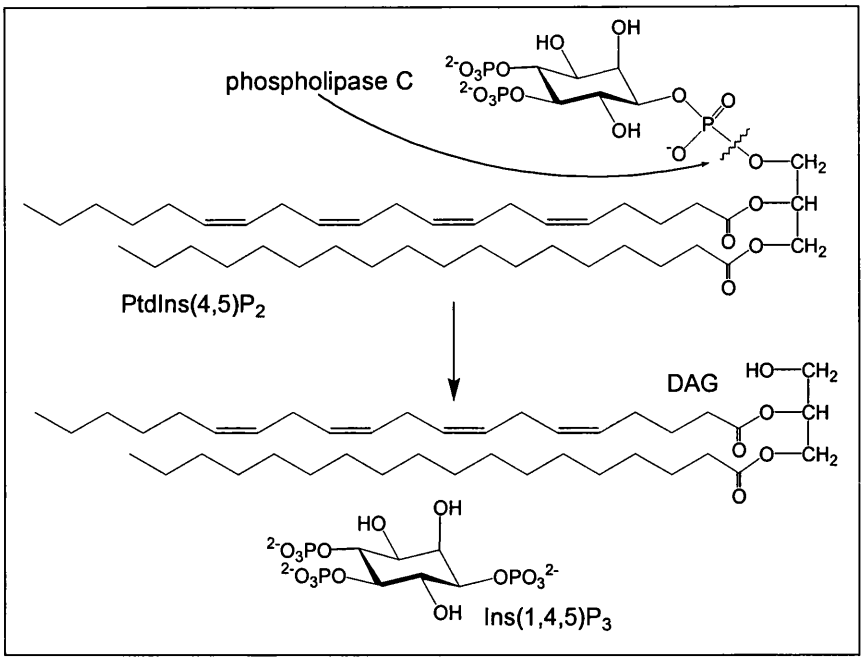


Figure 1.4: Hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C produces inositol 1,4,5-trisphosphate and diacylglycerol.

Phosphatidylinositol (PtdIns) is synthesised from *myo*-inositol and CDP-diacylglycerol by a reaction that may take place in the endoplasmic reticulum. Recent evidence suggests that it may also be synthesized on the plasma membrane. In mammalian cells, *myo*-inositol is either taken up from food or is provided by the dephosphorylation of inositol phosphates. It is worth noting that *myo*-inositol cannot cross the blood-brain barrier, so the brain must rely upon recycled *myo*-inositol. Plants can synthesize D-*myo*-inositol-3-phosphate from D-glucose-6-phosphate.

The other substrate, CDP-DAG is formed from CTP and phosphatidic acid (PtdA), the latter originating either from *de novo* synthesized DAG, or from DAG produced by phospholipid hydrolysis by PLC. Once PtdIns has been synthesized it may be phosphorylated by PI and PIP kinases at the various positions of the inositol ring to give PtdIns(4,5)P_2.

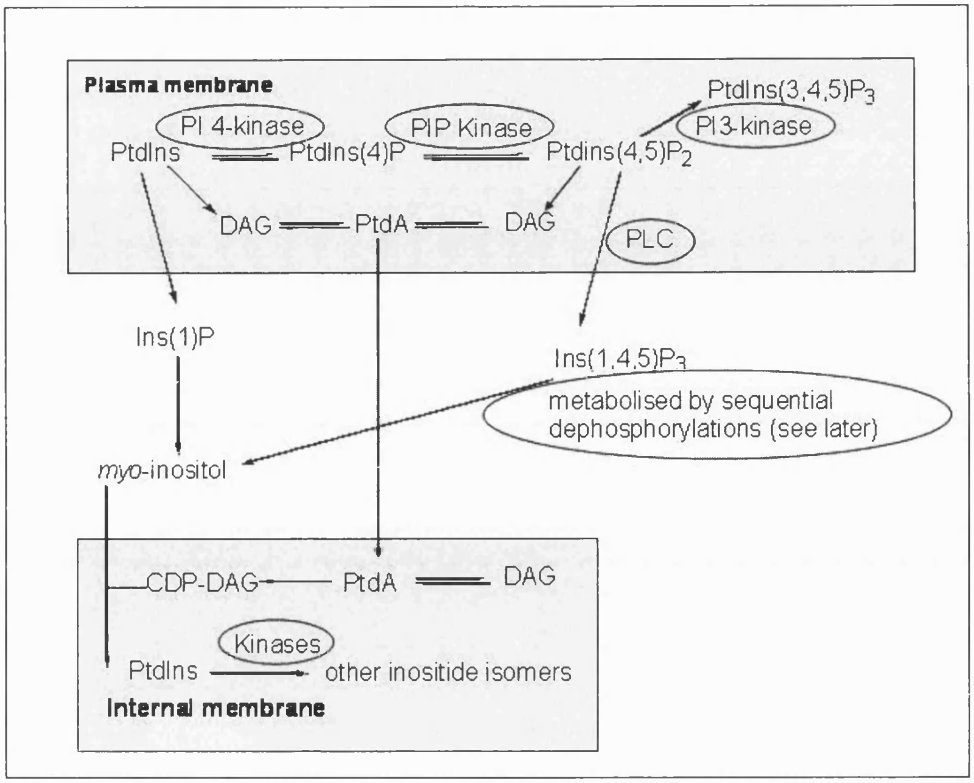


Figure 1.5: Summary of the synthesis of phosphatidylinositol 4,5-bisphosphate.

1.3 Phospholipase C

There are eleven distinct isoforms of phosphoinositide-specific PLC, which are grouped into four subfamilies (β, γ, δ, and ε). This diverse group of enzymes differ in structure and tissue distribution. As mentioned earlier, PLC hydrolyses PtdIns(4,5)P_2 to Ins(1,4,5)P_3 and DAG in response to the activation of more than a hundred different cell surface receptors (Table 1.1).

All PLC isoforms contain X and Y domains, which form the catalytic core, as well as various combinations of regulatory domains that are common to many other signalling proteins. The presence of distinct regulatory domains renders them susceptible to different modes of activation, which is likely to be a highly regulated process. The regulation of phosphoinositide specific PLC has been extensively reviewed by Rhee[6].

G PROTEIN LINKED RECEPTORS VIA PLCβ	TYROSINE KINASE LINKED RECEPTORS VIA PLCγ
α 1-Adrenergic	Epidermal growth factor receptor
Muscarinic m1, m3, m5	Platelet-derived growth factor receptor
Purinergic P2y, P2u, P2t	Fibroblast growth factor receptor
Serotonin 5HT 1 C	T cell receptor
H1	
Glucagon	
Cholecystokinin	
Vasopressin	
Oxytocin	
Angiotensin II	
Thrombin	
Bombesin	
Vasoactive intestinal peptide	
Bradykinin	
Platelet-activating factor	

Table 1.1: Plasma Membrane Receptors Increasing Intracellular Ca^{2+}[7]

1.4 G-protein-linked receptors

As mentioned earlier, activation by G-protein linked receptors is one of the two major mechanisms for the formation of Ins(1,4,5)P_3. They are coupled to an energy-requiring (GTP) transducing mechanism which activates PLC to hydrolyse the lipid precursor PtdIns(4,5)P_2 to give both DAG and Ins(1,4,5)P_3.

In general, these receptors contain seven regions of approximately twenty two to twenty four amino acids which form hydrophobic α-helices spanning the plasma membrane. The classes of G-protein involved in signal transduction from cell surface receptors are known as trimeric or heterotrimeric G-proteins. As their name suggests they are composed of three subunits α, β and γ.

When at rest the three subunits are bound together and a molecule of GDP is bound to the α-subunit. When the receptor is activated by an agonist, a conformational change in the receptor is induced. This change stimulates the dissociation of bound GDP from the α-subunit. GTP then rapidly binds to the α-subunit (Gα). The Gα-GTP complex dissociates from the G$\beta\gamma$-complex and diffuses through the membrane until it encounters a molecule of PLCβ which it activates to produce Ins(1,4,5)P_3 and DAG (Figure 1.6).

Activation of PLC ceases when the GTP associated with the G-protein is hydrolysed to GDP by the intrinsic GTP-ase activity of the Gα subunit. The Gα-GDP then dissociates from PLC and rejoins the G$\beta\gamma$ complex therefore returning the G-protein to rest.

1.5 Tyrosine kinase-linked receptors.

The other pathway responsible for stimulating the release of Ins(1,4,5)P_3 begins with the tyrosine kinase receptors. The ligand-binding domain is found on the extracellular side of the membrane, with usually a single span of the membrane leading to the catalytic domain on the cytoplasmic side. Like the G-protein linked receptors, these receptors also require energy as ATP is consumed not only as the two receptors interact (autophosphorylation) but also during the subsequent phosphorylation of PLCγ. The phosphoryl groups added to the receptor itself create binding sites for the SH2 domains of PLCγ and so allow diffusion of PLCγ to the membrane where it can associate with the receptor. Once association has occurred, phosphorylation activates the enzyme to produce Ins(1,4,5)P_3 and DAG (figure 1.7).

DAG and Ins(1,4,5)P_3 both have roles as second messengers. DAG can be degraded by lipases or it can be phosphorylated by DAG-kinases to phosphatidic acid (PtdOH). More importantly for cell signalling, DAG activates another family of enzymes, phospho-kinase C, which results in a cascade of phosphorylations of other proteins and enzymes. Massive amplification of the original signal occurs because one molecule of phospholipase C produces many molecules of Ins(1,4,5)P_3 and DAG.

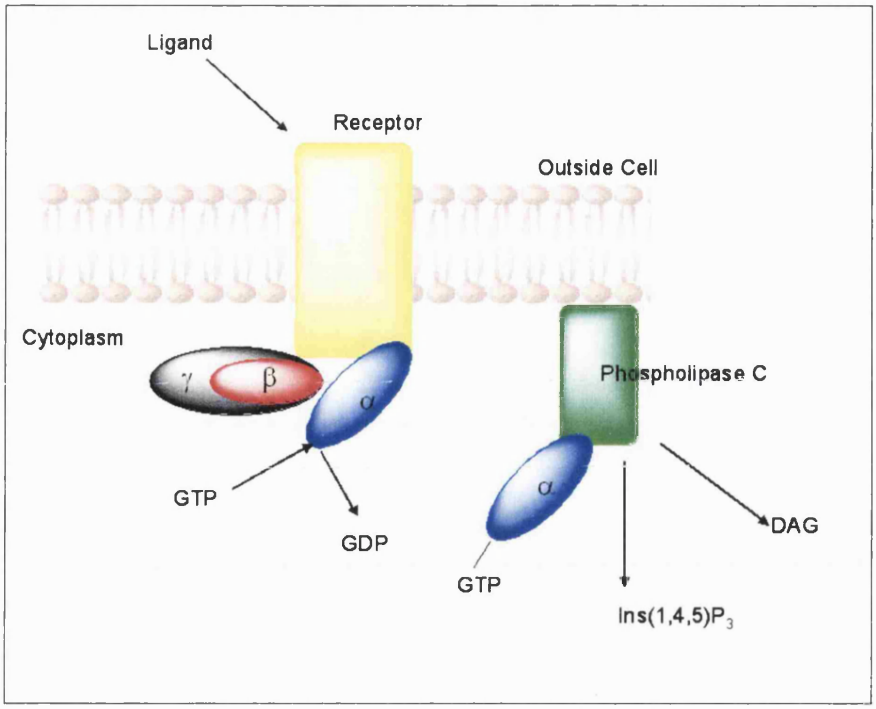


Figure 1.6: Activation of a trimeric G-protein.

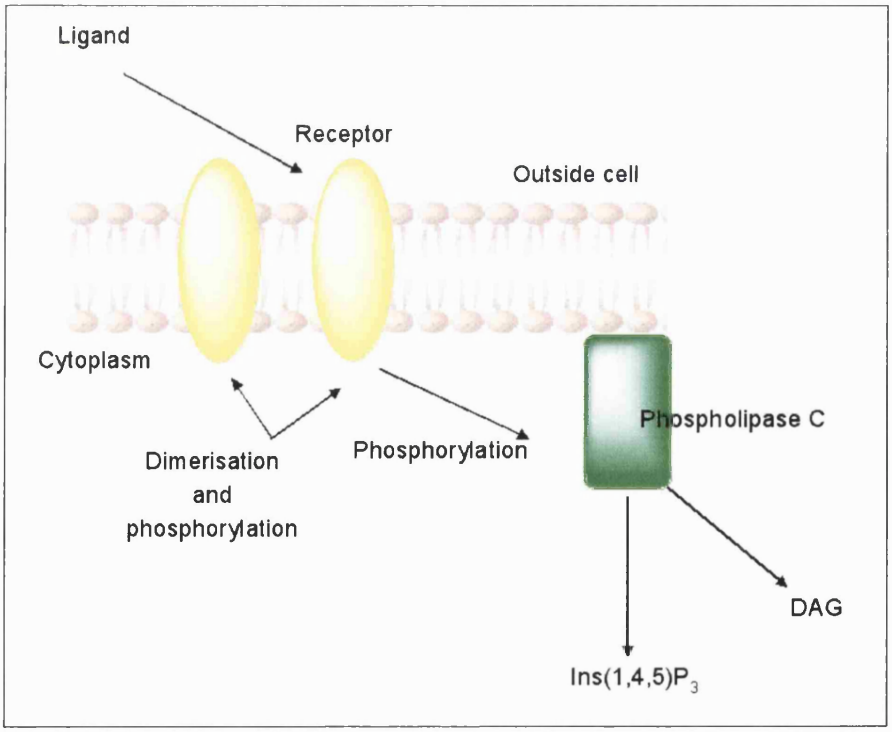


Figure 1.7: Activation of a tyrosine kinase receptor.

1.6 The Ins(1,4,5)P_3 Receptor, its structure and regulation

The Ins(1,4,5)P_3 receptor is a tetramer, composed of four subunits surrounding an anionic pore. Each subunit is a large protein with a molecular mass of about 300 kDa. Each subunit has three functionally distinct regions as follows.

- Ins(1,4,5)P_3 binding site domain at the N-terminal region.
- Six transmembrane helices towards the C-terminal which forms the ion channel pore.
- Large regulatory domain separating the two regions.

The transmembrane topology is thought to be as shown in Figure 1.8 and discussed further below.

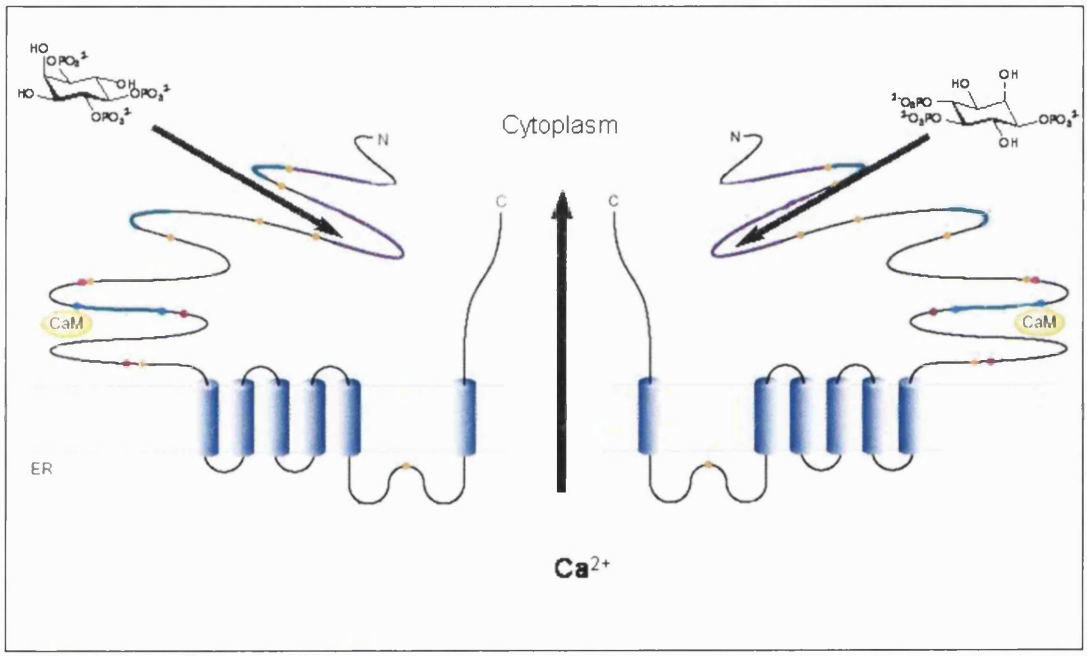


Figure 1.8: Cross-section of a generalised Ins(1,4,5)P_3 receptor. Ca^{2+} binding sites are depicted as orange circles, ATP-binding sites are shown as dark red circles, and phosphorylation sites are shown as cyan circles. Adapted from Thrower *et al.*[8]

The Ins(1,4,5)P_3 receptor is a ligand gated Ca^{2+} channel, which responds to Ins(1,4,5)P_3 produced when the cell surface receptors are activated. When Ins(1,4,5)P_3 binds to at least three and possibly all four subunits of the receptor (Figure 1.9), Ins(1,4,5)P_3R undergoes a conformational change that opens the Ca^{2+} ion channel[9].

This event occurs relatively quickly and the receptor returns to its original state very rapidly.

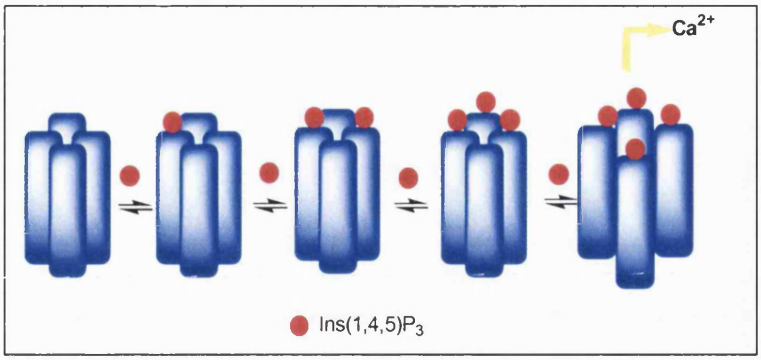


Figure 1.9: Activation of the $Ins(1,4,5)P_3$ receptor by $Ins(1,4,5)P_3$.

A recent report involving electron microscopy of the $Ins(1,4,5)P_3R$ particles revealed two distinct structures with 4-fold symmetry; a windmill structure and a square structure (figure 1.10). They showed that Ca^{2+} reversibly promoted a transition from the square structure to the windmill structure with relocation of four peripheral $Ins(1,4,5)P_3$-binding domains[10].

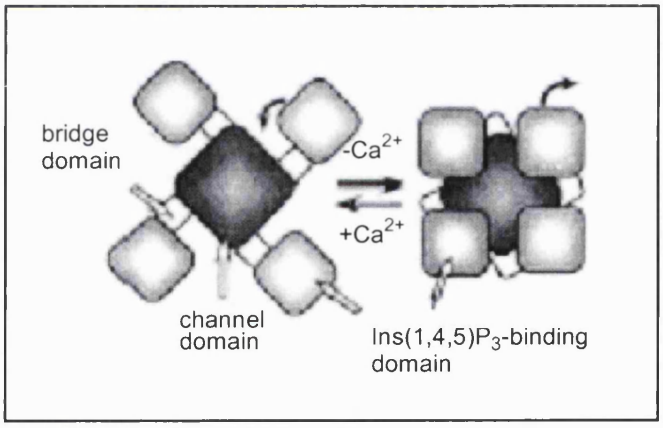


Figure 1.10: Proposed model for structural rearrangement within the $Ins(1,4,5)P_3R$. $Ins(1,4,5)P_3$ binding domain (light grey), channel domain (grey), and bridge domain (white). Black arrows indicate plausible domain movements.

Three distinct $Ins(1,4,5)P_3$ isoforms have been identified termed IP_3R1, IP_3R2 and IP_3R3. For a recent review of the isoform-specific functions see Thrower *et al*[8]. All three isoforms share 60–70% of the same homology with each other, partial homology with the ryanodine receptor and no significant homology with voltage-gated Ca^{2+} channels. All cells contain multiple receptor isoforms but one isoform may be predominant in a particular tissue or cell type.

FAMILY	PREDOMINANT TISSUE DISTRIBUTION
IP_3R1	Cerebellum, uterus, peripheral tissue
IP_3R2	Spinal cord, lung, hepatocytes, testis, spleen
IP_3R3	Brain, gastrointestinal tract, kidney, pancreatic islets

Table 1.2: Location of Ins(1,4,5)P_3 receptor isoforms.

In general Ins(1,4,5)P_3 receptors are located in the membranes of the ER; however in some tissues the Ins(1,4,5)P_3 receptors have been found in the plasma membrane and nuclei[11]. This could indicate that in some cell types, Ins(1,4,5)P_3 receptors may have a role in Ca^{2+} entry from outside the cell.

The study of Ins(1,4,5)P_3 signalling has been complicated further by the fact that the different Ins(1,4,5)P_3 receptor isoforms can combine to form mixed tetramers therefore providing a mechanism for generating greater diversity of Ins(1,4,5)P_3 receptors[12].

1.6.1 The Ins(1,4,5)P_3 receptor binding domain

Early studies indicated that the large N-terminal region of the Ins(1,4,5)P_3 receptor incorporates six hundred and fifty amino acid residues, thought to contain the sequence that forms the 3-D structure of the Ins(1,4,5)P_3 binding sites. Finally the Ins(1,4,5)P_3 binding site of the Ins(1,4,5)P_3R is thought to have positive charges to facilitate ionic interaction with negative charges on the three phosphate groups of Ins(1,4,5)P_3. More recent studies, involving deletion mutagenesis of the N-terminal region of the Ins(1,4,5)P_3R, have mapped the amino acid residues essential for ligand binding activity. Three basic residues, Arg 265, Lys 508 and Arg 511 are critical for specific binding, while Arg 568 is important in regulating the binding specificity for various inositol polyphosphates[13].

The binding affinity of Ins(1,4,5)P_3 for the N-terminal segment varies between different receptor isoforms. Two different groups have found different rank orders for the binding affinity of Ins(1,4,5)P_3 to the receptor isoforms[14-16].

1.6.2 The Ins(1,4,5)P_3 receptor transmembrane domain

Recently, it has been experimentally proven that there are six transmembrane spanning regions, thus projecting the C-terminus of the protein into the cytoplasm[17]. A hydrophobic region between the fifth and sixth transmembrane domain has been proposed to form a hairpin loop that is partially embedded in the membrane. This may function as part of the ion-conducting pore (P-domain) of the Ca^{2+} channel. Negatively charged amino acids around the P-domain may concentrate Ca^{2+} ions around the pore.

Furthermore, the transmembrane-spanning segments are also thought to be required for the oligomerization of the four Ins(1,4,5)P_3 receptor subunits.

1.6.3 The Ins(1,4,5)P_3 receptor regulatory domain

The regulatory domain separates the Ins(1,4,5)P_3 binding domain from the transmembrane domain. As shown in figure 1.8, the domain contains binding sites for various modulators of the channel such as ATP, Ca^{2+}, Calmodulin (CAM) and phosphorylation sites for several protein kinases.

The homology among different Ins(1,4,5)P_3R subtypes is the lowest in the regulatory domain, suggesting the operation of differential regulation in different subtypes[18].

1.6.4 Three dimensional structure of the type 1 Ins(1,4,5)P_3 receptor.

The first 3-D structure of IP_3R1 has very recently been reported [19]. Purified receptors were shown to be functional by Ins(1,4,5)P_3 binding and Ca^{2+} efflux assays were used to obtain the images. The 24 Å resolution structure takes the shape of an uneven dumbbell with one end larger than the other. The bulky large end probably corresponds to the cytoplasmic domain. The smaller end has structural features indicative of the membrane-spanning domain. A central opening in this domain, which is occluded on the cytoplasmic half, outlines a pathway for Ca^{2+} flow in the open state of the channel.

1.7 Ins(1,4,5)P_3 induced Ca^{2+} release

A feature of the Ins(1,4,5)P_3R is its Ca^{2+} induced Ca^{2+} release (CICR) property. This is caused by a positive effect of Ca^{2+} at the low concentration range, (above resting cytoplasmic Ca^{2+}) on channel opening, while high Ca^{2+} levels (μM range) cause inactivation.

Ins(1,4,5)P_3 determines the concentration of Ca^{2+} at which the Ins(1,4,5)P_3 channels open. Ca^{2+} increases without the presence of Ins(1,4,5)P_3 cannot activate channel opening. Ins(1,4,5)P_3 concentration does not necessarily determine the amount of Ca^{2+} that is released, rather it is determined by the amount of Ca^{2+} in the Ca^{2+} store and the inactivation of the channel by Ca^{2+} and Ins(1,4,5)P_3.

1.8 Capacitative Ca^{2+} entry

When internal Ca^{2+} stores are depleted, Ca^{2+} influx through the plasma membrane occurs, this is known as capacitative Ca^{2+} entry or store operated Ca^{2+} influx channel (SOC). This idea was introduced when Ca^{2+} influx was found to be increased even in the absence of increased Ins(1,4,5)P_3 levels after emptying of the intracellular Ins(1,4,5)P_3 sensitive Ca^{2+} stores. Two major questions need to be answered in the field of capacitative Ca^{2+} entry;

1. What is the mechanism by which the depleted stores signal the Ca^{2+} entry channel?
2. What is the molecular entity that is responsible for Ca^{2+} entry through SOC?

Models have been proposed to explain Ca^{2+} influx. One model hypothesizes that a diffusible molecule is released after Ca^{2+} stores are depleted, and that it stimulates Ca^{2+} influx via Ca^{2+} channels on the plasma membrane. Numerous second messengers have been proposed to initiate Ca^{2+} influx, including small G proteins, pertussis toxin-sensitive G proteins, cGMP, various lipids, tyrosine phosphorylation and Ins(1,4,5)P_3. The most interesting candidate second messenger is Ca^{2+} influx factor (CIF). It has been isolated as an acid extract from Jurkat T cells and partially characterised as a pH stable small anionic phosphorylated molecule that induces Ca^{2+} influx[20]. CIF is probably released or generated from the ER or adjacent regions after Ins(1,4,5)P_3 induces Ca^{2+} release from stores.

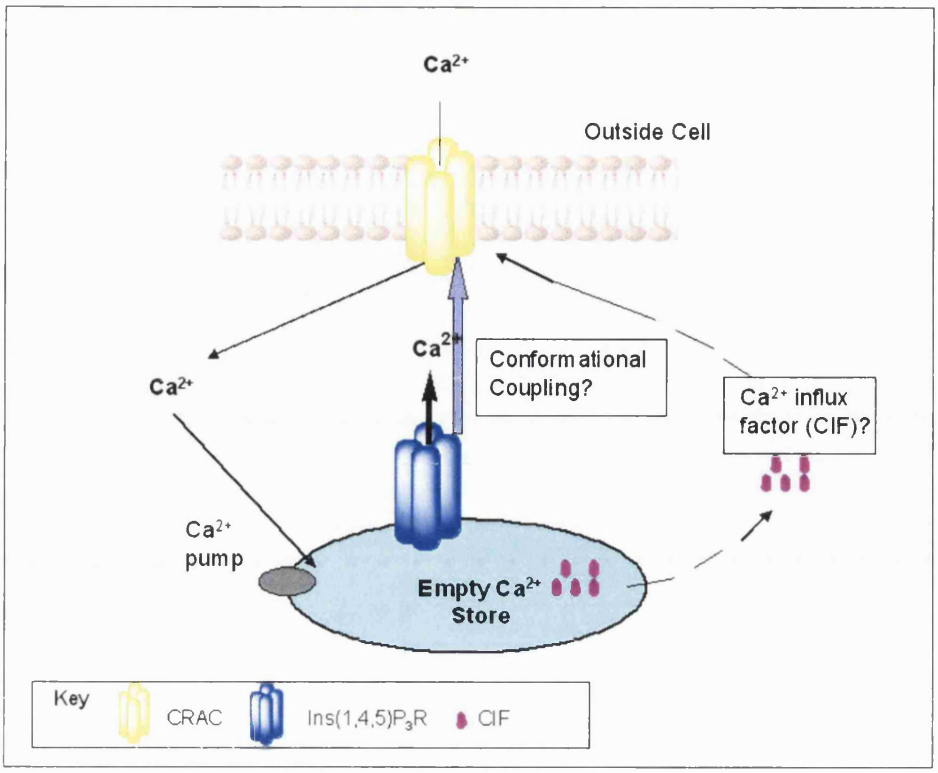


Figure 1.11: Capacitative Ca^{2+} entry. The depletion of endoplasmic reticulum Ca^{2+} stimulates the entry of Ca^{2+} through the plasma membrane. Two theories are depicted in the figure; conformational coupling and a diffusible Ca^{2+} influx factor (CIF).

The second model postulates that internal Ca^{2+} release channels conformationally couple to the plasma membrane Ca^{2+} channel, SOC also referred to as CRAC (Ca^{2+} release activated Ca^{2+} channels). The idea is that the cytoplasmic head of the Ins(1,4,5)P_3R communicates with two Ca^{2+} channels, one in the ER and the other in the plasma membrane. Ins(1,4,5)P_3 binding will induce a conformational change which creates the channel that releases Ca^{2+} from the store. Once the store is empty the receptor changes the conformation leading to the opening of the CRAC on the plasma membrane[21]. In summary, the conformational coupling hypothesis considers that Ins(1,4,5)P_3R controls the mobilisation of both the internal and external Ca^{2+}. These two dominant theories of capacitative Ca^{2+} entry, conformational coupling and CIF are summarised schematically in figure 1.11.

1.9 Metabolism of Inositol phosphates

Once produced, $Ins(1,4,5)P_3$ is metabolized extremely quickly, in order to terminate its action and return the cell to rest. Two enzymes catalyse the conversion of $Ins(1,4,5)P_3$ in cells. 5-Phosphatase cleaves the phosphate group at position 5 and 3-kinase catalyses ATP-dependent phosphorylation of $Ins(1,4,5)P_3$ at position 3. The important features of $Ins(1,4,5)P_3$ metabolism are detailed in figure 1.12, however this is a somewhat simplistic view of what is a complex system of metabolites and metabolising enzymes.

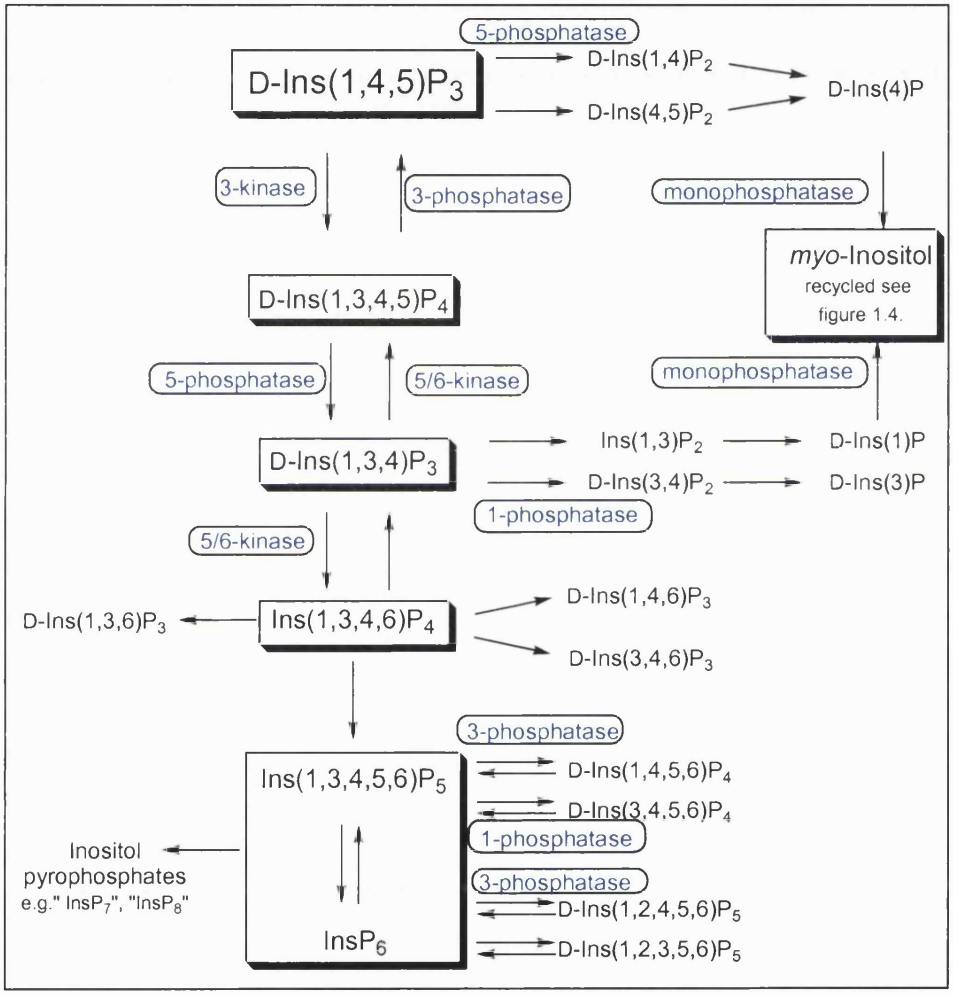


Figure 1.12: Metabolism of inositol phosphates. Adapted from Potter and Lampe[22].

$Ins(1,4,5)P_3$ is dephosphorylated at position 5 to produce $Ins(1,4)P_2$, which in turn, is further dephosphorylated at position 1 to give Ins(4)P. Several 5-phosphatases

have been indentified. Some of these enzymes are involved in the dephosphorylation of inositol lipids rather than inositol phosphates.

$Ins(1,4,5)P_3$ can be phosphorylated at the 3-position by $Ins(1,4,5)P_3$ 3-kinase to form $Ins(1,3,4,5)P_4$. Three isoforms (A, B and C) of 3-kinase have been cloned[23]. $Ins(1,3,4,5)P_4$ is then metabolised by the same 5-phosphatase as $Ins(1,4,5)P_3$ to give $Ins(1,3,4)P_3$. This can then be dephosphorylated via two different routes, via $Ins(3,4)P_2$ and $Ins(1,3)P_2$. Although many of these metabolic intermediates are believed to be biologically inactive, it is quite possible that some are involved in the synthetic routes by which higher phosphates, such as $Ins(1,3,4,5,6)P_5$ and $InsP_6$ are synthesised.

1.10 Structure-Activity Relationships at $Ins(1,4,5)P_3$ Receptors

Over the last couple of decades there has been considerable interest in establishing structure-activity relationships for the $Ins(1,4,5)P_3$ receptor. This has recently been covered in two comprehensive reviews [22],[24] and only a short summary will be presented here. The interaction of $Ins(1,4,5)P_3$ with the receptor is highly stereospecific, with the D-isomer being over 1000-fold more potent than the L-isomer. The relative importance of the functional groups $Ins(1,4,5)P_3$ are summarised in figure 1.13. Each ring position of $Ins(1,4,5)P_3$ will now be considered.

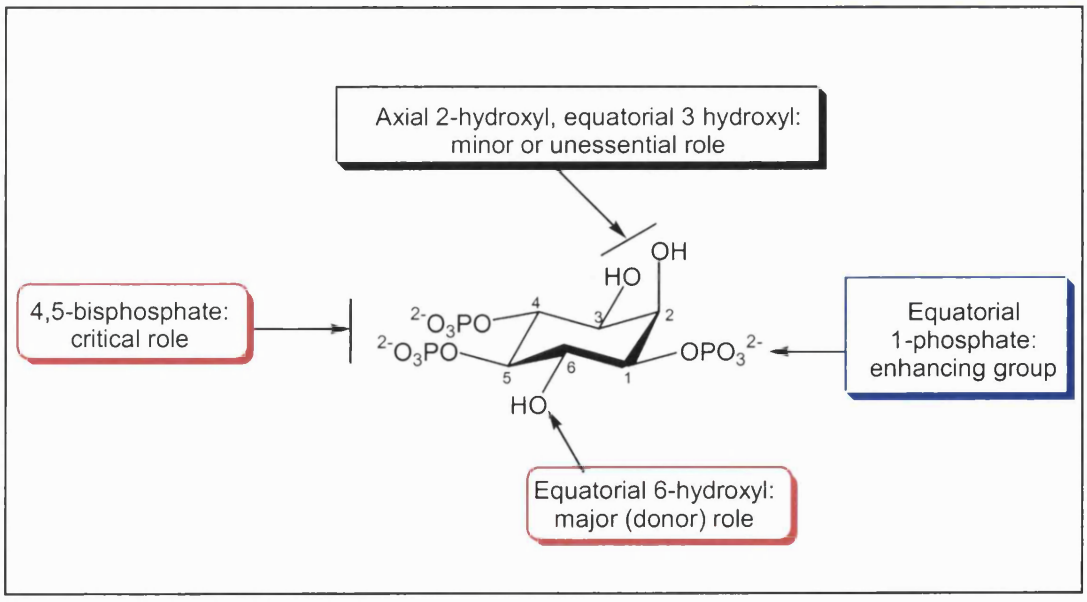


Figure 1.13: Relative importance of the functional groups of $Ins(1,4,5)P_3$ to its Ca^{2+} binding and mobilising properties.

Position 1

The equatorial 1-phosphate is less important than the 4- and 5-phosphates. However, as Ins(4,5)P_2 binds considerably more weakly than Ins(1,4,5)P_3, the 1-phosphate must contribute to receptor binding[24]. The 1-phosphate may be replaced with an axial phosphate or a phosphate more distant from the ring without major loss of activity. Large groups are also tolerated and this fact has been exploited by attaching photoaffinity ligands and fluorescent labels to the 1-phosphate of Ins(1,4,5)P_3.

Inoue *et al.*[25] recently described a 1-position modified Ins(1,4,5)P_3 analogue with remarkable binding affinity. Modification of this 1-phosphate with the dye molecule malachite green (Figure 1.14) resulted in a compound with a K_d of 1.17 nM in the Ins(1,4,5)P_3-binding domain of human type-1 Ins(1,4,5)P_3 receptor. In the same study the corresponding binding of Ins(1,4,5)P_3 (K_d 195nM) was found to be 167-fold weaker. Since then this group has synthesised more analogues (Figure 1.14) to investigate the importance of steric factors and charges[26]. The authors concluded that linking a hydrophobic moiety to the 1-phosphate of Ins(1,4,5)P_3 enhances the binding of Ins(1,4,5)P_3 analogues to the receptor with considerable latitude of substituent structure. For strong interaction with the receptor, a cationic charge adjacent to the 1-phosphate of Ins(1,4,5)P_3 appears to be important.

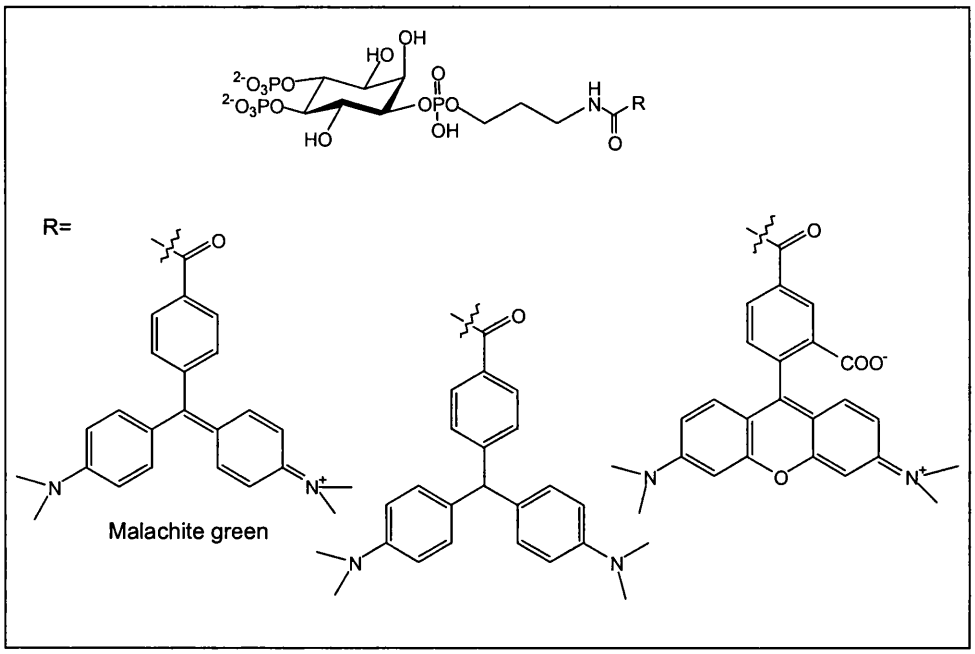


Figure 1.14: Recently reported 1 position modified Ins(1,4,5)P_3 analogues.

Position 2

The axial 2-hydroxyl is the least important part of Ins(1,4,5)P_3. It can be deleted, changed to equatorial or replaced with fluorine with only slight effect on activity, therefore suggesting that it is not very important for binding or for Ca^{2+} release [22].

Position 3

The equatorial 3-hydroxyl enhances binding, but is less important than the 6-hydroxyl. Stereochemical inversion of C-3 causes loss of activity[27], while increasing the steric bulk of the equatorial 3-position produces a series of Ins(1,4,5)P_3 analogues with progressively decreasing activity at the Ins(1,4,5)P_3R. However D-3-C-trifluoro-methyl-*myo*-inositol 1,4,5-trisphosphate, which retains the equatorial 3-hydroxyl, but has an axial 3-trifluoromethyl group, has almost the same potency as Ins(1,4,5)P_3. This suggests that bulky substituents in the 3-axial position can be efficiently accommodated in the Ins(1,4,5)P_3R[22].

Positions 4 and 5

The 4,5-bisphosphate is the most crucial part of Ins(1,4,5)P_3 as it is essential for activity. The two phosphate groups are vicinal and diequatorial. It has recently been suggested that the 5-phosphate is the dominant partner in receptor interaction as replacement of the 4-phosphate with phosphorothioate has less effect than replacing the 5-phosphate[24].

Position 6

The hydroxyl group at position 6 is the most sensitive of the three unphosphorylated hydroxyl groups in the cyclitol to modification. It is thought to make a major contribution as 6-deoxy- inositol 1,4,5-trisphosphate was found to be a very weak agonist at the Ins(1,4,5)P_3R[28]. The 6-hydroxyl group may be able to hydrogen bond to an amino acid residue on the receptor. Alternatively it may form an intramolecular hydrogen bond to the 1- or 5-phosphate group. Either way, the hydrogen bonds may be important in defining the receptor recognising properties of Ins(1,4,5)P_3. The receptor probably cannot tolerate steric bulk at this position as substitution with fluorine and phosphate greatly reduces activity.

1.10.1 Partial agonists

A partial agonist is an agent that will occupy the receptor but will produce a response somewhat less than the maximum possible. Some Ins(1,4,5)P_3 analogues can bind to the Ins(1,4,5)P_3R but are not able to mobilise the full Ca^{2+} stores, these are referred to as class I partial agonists. The first analogue found to exhibit this activity was the naturally occurring Ins(1,3,4,6)P_4 (Figure 1.15)[29]. It is thought to adopt a binding orientation which efficiently mimics Ins(1,4,5)P_3 with the exception of presenting an axial 3-phosphate and an additional phosphate in the equatorial 2-position.

Phosphorothioate groups at positions usually substituted with phosphate groups were prepared originally as they were stable to the metabolising enzymes Ins(1,4,5)P_3 3-kinase and Ins(1,4,5)P_3 5-phosphatase. Two of these analogues from the Potter group D-6-deoxy-Ins(1,4,5)PS_3 (**7**) and L-*chiro*-Ins(2,3,5)PS_3 (Figure 1.15) have been shown to be partial agonists[22]. These compounds are C-3- or C-6- modified analogues of Ins(1,4,5)P_3, respectively, in addition to carrying phosphothioate groups rather than phosphates in the 1-, 4- and 5-positions. The replacement of oxygen with sulphur to form the phosphorothioate group results in considerable disturbance in charge distribution with an increased negative charge on the sulphur and reduced negative charge on the remaining oxygen. These changes in charge distribution appear to have subtle effects in the interactions of the compounds with the receptor[30].

Studies then concentrated on finding the key elements to partial agonist activity. It was noted that modifications of the 3-hydroxyl resulted in a decreased efficacy. One of the best partial agonists described so far is the 3-fluorinated 4,5-phosphorothioate analogue of Ins(1,4,5)P_3 [3F-Ins(1)P(4,5)PS_2, (Figure 1.15)][30], which retained a relatively high affinity for the Ins(1,4,5)P_3 binding site, accompanied by a significant loss in both potency and intrinsic activity for Ca^{2+} mobilisation. The retention of the 1-phosphate group probably enhances the interaction with the Ins(1,4,5)P_3R binding pocket.

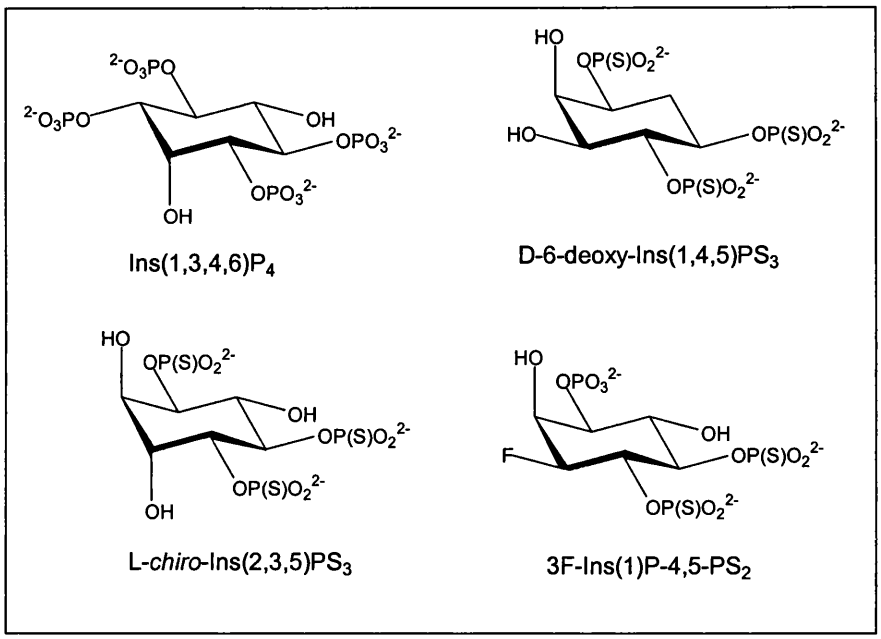


Figure 1.15: Class I partial agonists at Ins(1,4,5)P_3 receptors.

Some analogues can mobilise the full Ca^{2+} store, however at a much slower rate than Ins(1,4,5)P_3; this has been defined as class II partial agonist[31]. These analogues include a series of 3-position modified Ins(1,4,5)P_3 analogues and Ins(2,4,5)P_3 (Figure 1.16).

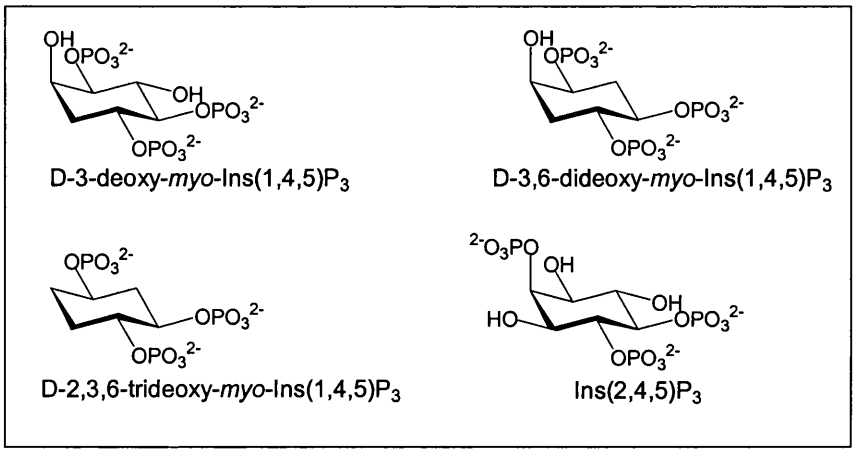


Figure 1.16: Class II partial agonists at Ins(1,4,5)P_3 receptors.

1.10.2 Antagonists

No good antagonist of the Ins(1,4,5)P_3R has been found in spite of many efforts using the inositol backbone to create inhibitory analogues. For several years heparin and decavanadate were the only known Ins(1,4,5)P_3R antagonists. Indeed they can stop Ins(1,4,5)P_3 induced Ca^{2+} release in cell free systems or when injected into cells. However they are both non-selective and they can bind to a number of proteins to inhibit their functions. They are therefore not ideal for use as pharmacological agents.

Xestospongins have recently been isolated from the Australian sponges of *Xestspongia* and were shown to inhibit Ins(1,4,5)P_3-induced Ca^{2+} release in cell free systems as well as in cells. Xestospongin C (Figure 1.17) was the most potent. However xestospongins also block ryanodine receptors, but only at thirty times higher concentrations[24].

Another antagonist at the Ins(1,4,5)P_3R is 2-aminoethoxydiphenylborate (2-APB)(Figure 1.17). It has been found to inhibit Ins(1,4,5)P_3 action in platelets and neutrophils. Unlike xestospongins, 2-APB does not appear to block Ca^{2+} release from the ryanodine receptor[32].

Both xestospongins and 2-APB are considered to have the advantage of being cell-permeable, non-competitive agonists at the Ins(1,4,5)P_3R, as they are thought to bind to the Ins(1,4,5)P_3R at a site other than the Ins(1,4,5)P_3 binding site.

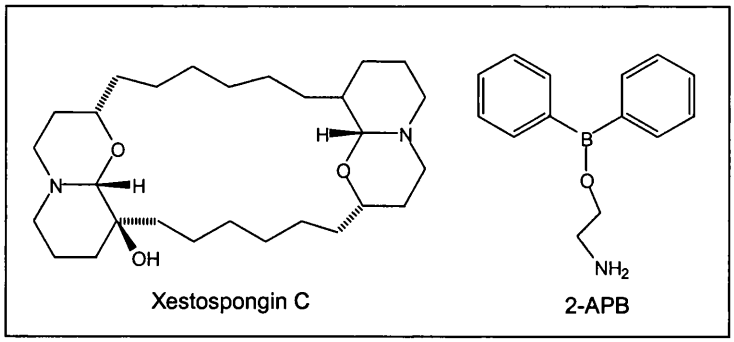


Figure 1.17: The structure of two antagonists at the Ins(1,4,5)P_3 receptor.

1.11 Binding Domains

The effects of phosphoinositides are mediated primarily by direct interaction with a large number of downstream effector proteins, and these protein-lipid interactions involve numerous, specific lipid-binding modules, including the pleckstrin homology (PH), FYVE and ENTH domains (recently reviewed in [33]) and PX domain[34]. Since there are x-ray structures of Ins(1,4,5)P_3 to the PH domains, a short summary about PH domains follows.

1.11.1 Structure of the PH domain

The pleckstrin homology (PH) domain is a structural protein of around one hundred amino acids that was first identified in 1993, occurring as an internal repeat in the phosphoprotein pleckstrin. Since then it has been found in more than one hundred different proteins involved in cellular signalling, cytoskeletal organisation, regulation of intracellular membrane transport and modification of membrane phospholipids. Examples include some protein kinases, all phospholipase C isoforms, and the phosphoinositide 3-kinase p110γ subunit[35].

Although the primary sequence of PH domains varies considerably, their secondary structure is quite similar, this being one of the criteria for a PH domain. It is formed by a seven stranded antiparallel β-sheet with a strong bend that results in an orthogonal sandwich. There are six loops connecting the β-strands, three of these have been termed variable loops as they are variable in length and sequence. The domain has a characteristic C-terminal α-helix which blocks one end of the twisted sheet.

The PH domain has been found in proteins expressed in mammals and unicellular eukaryotes but not in plants or bacteria. Their amino acid sequences are diverse, the only distinguishing characteristic of the PH domain sequence is a single almost invariant tryptophan residue in the C terminus of the domain. One side of the domain, including the variable loops, is a positively charged surface, made up of lysines, arginines and histidines, while the opposite face, including the α-helix, is enriched in acidic residues[36].

1.11.2 Function

The majority of PH domain-containing proteins appear to have a functional requirement to be membrane associated. It is believed that PH domains function as membrane adapter/tethers, linking the host protein to the membrane surface often by binding to phosphoinositides. Most PH domains bind to phosphoinositides or inositol phosphates with a broad range of specificity and affinity, some of these are summarised in table 1.3.

PROTEIN	LIGAND	AFFINITY
Pleckstrin	PtdIns(4,5)P_2	13.4 μM
β-Spectrin	g-PtdIns(4,5)P_2	40 μM
PLC-δ_1	g-PtdIns(4,5)P_2	0.46 μM
	PtdIns(4,5)P_2	1.7 μM
	D-Ins(1,4,5)P_3	210 nM
PLC-γ	Ins(1,3,4,5)P_4	1–2 μM
Dynamin	g-PtdIns(4,5)P_2	4.3 μM
Dynamin	D-Ins(1,4,5)P_3	1.23 mM

Table 1.3: Summary of proposed ligands for some PH domains[36].

Of particular interest is the interaction of PLC-δ_1 with Ins(1,4,5)P_3 and PtdIns(4,5)P_2. It is believed that PLC-δ_1 uses its PH domain to bind PtdIns(4,5)P_2 in the cell membrane. The catalytic domain of PLC then hydrolysises PtdIns(4,5)P_2, to give free Ins(1,4,5)P_3, which then inhibits the binding of further PtdIns(4,5)P_2 to the PH domain.

1.11.3 X-ray crystal structures of Ins(1,4,5)P_3

In 1995 two groups independently published x-ray crystal structures of Ins(1,4,5)P_3 bound to PH domains[37;38]. For the first time this enabled us to see the conformation of Ins(1,4,5)P_3 at one of its binding sites.

The x-ray structure of Ins(1,4,5)P_3 at the spectrin domain shows direct hydrogen bonds between Ins(1,4,5)P_3 and five amino acids. The 4- and 5-phosphate groups are

anchored by salt bridges to positively charged amino acids, and by hydrogen bonds to Tyr23 and Tyr69. The 1-phosphate forms a single hydrogen bond to Ser22, and is most likely to be exposed to solvent. The upper face of the molecule, including the area around the axial 2-hydroxyl group is also open to solvent[38]. (Figure 1.18)

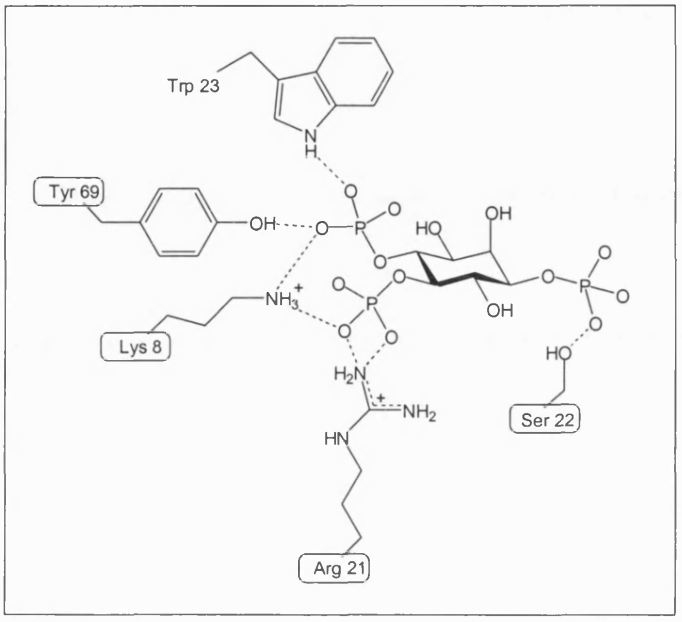


Figure 1.18: Schematic representation of the Ins(1,4,5)P_3 binding site of β-spectrin. Adapted from Hyvönen *et al.*[38]

The X-ray structure of Ins(1,4,5)P_3 at the PLC-δ_1 PH domain shows direct hydrogen bonding between the bound Ins(1,4,5)P_3 and seven amino acids. Two lysine chains (Lys30 and Lys57) each hydrogen bond with both the 4- and 5-phosphate groups of Ins(1,4,5)P_3. Additional interactions with the side chains of Arg56 plus additional hydrogen bonds via water molecules (not shown) ensure that the 5-phosphate of Ins(1,4,5)P_3 is completely buried. The 4-phosphate forms hydrogen bonds to the sidechains of Lys30, Lys32 and Lys57 and a water molecule. The 1-phosphate forms a single hydrogen bond to Trp36[37] (Figure 1.19).

The complex formed between PLC-δ_1 PH domain and Ins(1,4,5)P_3 is two hundred fold more stable than the complex between spectrin PH domain and Ins(1,4,5)P_3. This is not surprising as there are seven hydrogen bonds, involving five amino acids between Ins(1,4,5)P_3 and spectrin, while there are twelve hydrogen bonds, involving nine amino acids, between Ins(1,4,5)P_3 and PLC-δ_1 PH domain. In fact there are more interactions between the 5-phosphate and PLC-δ_1 PH domain than there are between the entire spectrin PH domain and Ins(1,4,5)P_3.

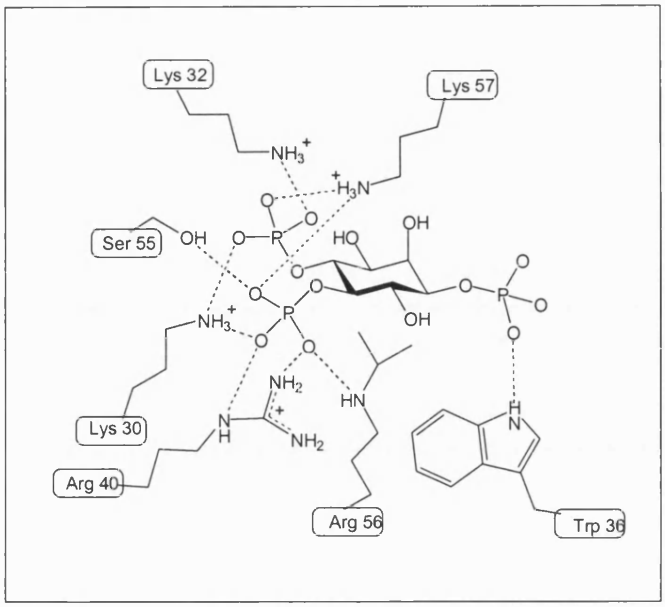


Figure 1.19: Schematic representation of the Ins(1,4,5)P_3 binding site of PLC-δ_1 PH domains. Adapted from Ferguson *et al.*[37]

These studies show the interaction of Ins(1,4,5)P_3 with the binding sites of PH domains. The results are in very good agreement with the conclusions reached about Ins(1,4,5)P_3R binding. However, the Ins(1,4,5)P_3 binding site of these PH domains do not necessarily resemble the Ins(1,4,5)P_3R. For example neither study showed any interactions of the 6-hydroxyl group of Ins(1,4,5)P_3 with the binding site of the PH domain, yet this is a very important feature in the Ins(1,4,5)P_3R. However, as many of the structure-activity relationships are the same for Ins(1,4,5)P_3R similar interactions and spatial relationships may be involved.

1.12 Radixin FERM domain

Radixin is a member of the ezrin/radixin/moesin (ERM) family of proteins, which function as cross-linkers between plasma membranes and actin filaments. The cross linking is regulated by PtdIns(4,5)P_2. Recently, the Hakoshima group published a x-ray crystal structure of Ins(1,4,5)P_3 bound to the radixin FERM domain[39]. The domain is responsible for membrane binding and it consists of three sub-domains.

Ins(1,4,5)P_3 binds in a basic cleft that is different from those found in PH domains. Three lysine chains (Lys60, Lys63 and Lys278) and one asparagine (Asn62) contact the three phosphate groups of Ins(1,4,5)P_3. The 1-phosphate group is exposed to

solvent, which enables PtdIns(4,5)P_2 to reach the binding site from the membrane surface. The 4-phosphate group intrudes into the cleft, whereas the 5-phosphate group resides at the molecular surface.

1.13 Adenophostin A and B

Adenophostin A and B (Figure 1.20) were first isolated in 1993 from the cultured broth of *Penicillium brevicompactum* SANK 11991 and SANK 12177[40]. They are potent Ins(1,4,5)P_3R agonists, which bind to Ins(1,4,5)P_3R, and induce Ca^{2+} release from Ins(1,4,5)P_3 sensitive stores.

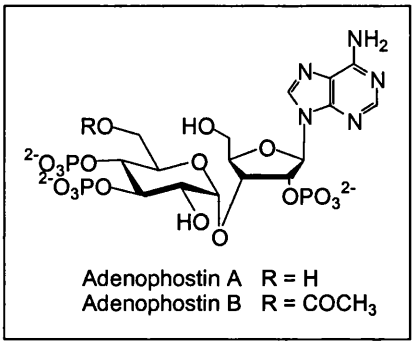


Figure 1.20: The structures of adenophostin A and adenophostin B

Their structures were first elucidated using a range of techniques including NMR, MS, elemental analysis and enzymatic degradation[41]. The structures were later confirmed by the first total synthesis of adenophostin A[42].

Adenophostins were originally found to have activities 100-fold more potent than Ins(1,4,5)P_3 at releasing Ca^{2+} from cerebellar microsomes[40]. Most subsequent papers only report a 10-fold increase in activity[43]. In addition, both adenophostins were found to inhibit [^{3}H]Ins(1,4,5)P_3 binding in a more potent manner than Ins(1,4,5)P_3 with a high positive cooperativity[44]. Their effect could be totally blocked by heparin.

Both adenophostins are resistant to phosphorylation and dephosphorylation by Ins(1,4,5)P_3-metabolising enzymes, Ins(1,4,5)P_3 3-kinase and Ins(1,4,5)P_3 5-phosphatase. They do not bind to Ins(1,3,4,5)P_4 receptors[44] and in both functional and radioligand binding assays of all three Ins(1,4,5)P_3R subtypes they bind with about 10-fold greater affinity than Ins(1,4,5)P_3[43].

When the structures of the adenophostins were first published it was surprising as they seemed so different from Ins(1,4,5)P_3. However on closer examination, the resemblances to Ins(1,4,5)P_3 became apparent. The 3" and 4" -bisphosphate and adjacent 2" -hydroxyl can be superimposed on the 5 and 4-equatorial bisphosphate and 6-hydroxyl

of Ins(1,4,5)P_3, respectively. This structural motif is an essential part of all Ins(1,4,5)P_3R agonists. A third phosphate group is not essential for activity in inositol phosphates but greatly enhances it. In adenophostins the third phosphate group is in the 2' position of the ribose ring; when removed it causes a 1000-fold reduction in activity[40]. The adenosine group of the adenophostins is the most surprising feature of these molecules. Finally, the charge distribution on the phosphate groups of adenophostin A at physiological pH is virtually identical with that in the equivalent phosphate groups of Ins(1,4,5)P_3; therefore, it may be concluded that the exceptional activity of adenophostin A cannot be explained by a difference in the ionisation state of its phosphate groups in comparison with those of Ins(1,4,5)P_3[45].

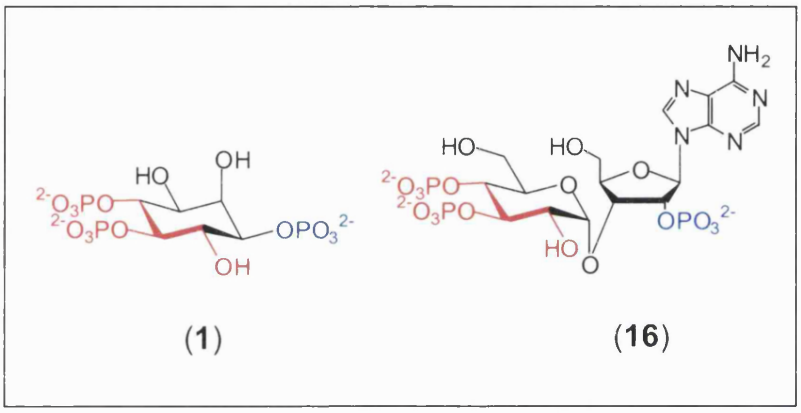


Figure 1.21: Structures of Ins(1,4,5)P_3 compared to Adenophostin A.

Although there are several common characteristics between Ins(1,4,5)P_3 and adenophostins, the exceptional ability of the adenophostins to mobilise Ca^{2+} through the Ins(1,4,5)P_3 receptor could not be explained. Thus further information about the structure activity relationships of adenophostins was needed, in particular about the adenine ring. In Chapter 2 and the rest of the thesis, we will look at the synthesis and the biological effects of adenophostin A and a range of analogues.

Chapter Two

Introduction

Chemical Synthesis
and
Biological effects of Adenophostin and analogues.

Chapter 2

2 Introduction-chemical synthesis and biological effects of adenophostin and analogues.

2.1 Synthetic Considerations

The synthesis of Ins(1,4,5)P_3 analogues from chiral carbohydrate starting materials is an attractive prospect as inositol phosphate chemistry is difficult and time consuming[22]. There is great attraction in focusing upon adenophostin A as a surrogate of this key signalling molecule, not only because of its exceptional potency but also because of the greater synthetic ease in dealing with readily available chiral carbohydrates as synthetic starting materials. The remainder of this chapter will introduce the synthesis of carbohydrate based analogues. Many published analogues will be reviewed and some structure activity relationships will be considered.

Several problems are inherent to the preparation of polyphosphates from either inositols or carbohydrate starting material.

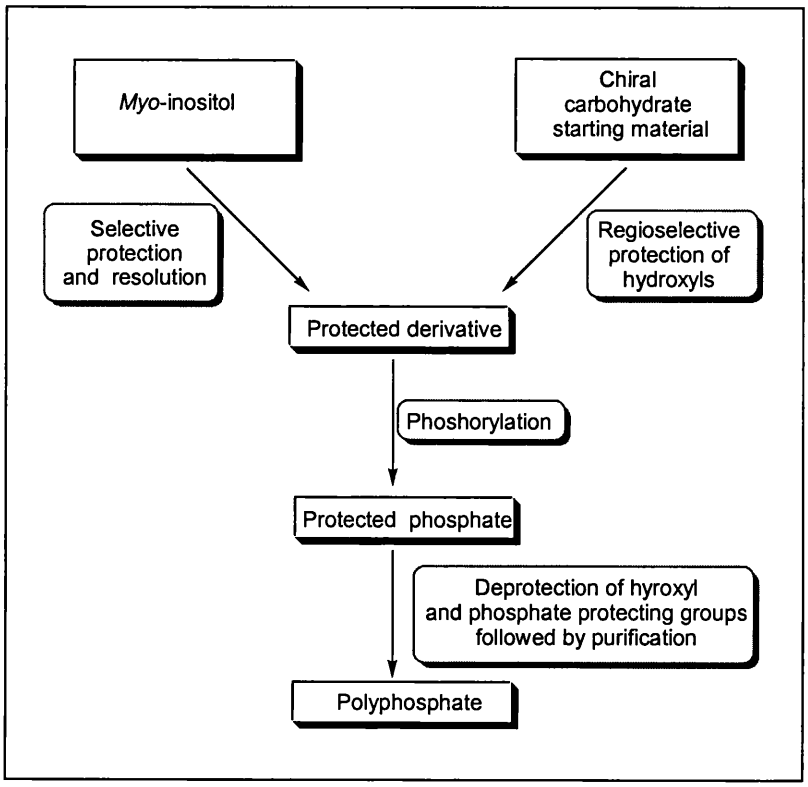


Figure 2.1: Steps in the synthesis of polyphosphates.

Protecting groups need to be chosen wisely in order to facilitate later incorporation of phosphate groups at selected positions. The most popular temporary and permanent hydroxyl protecting groups are acetyl, allyl, benzoyl, benzyl, butanediacetal (BDA), isopropylidene, *p*-methoxybenzyl, pivaloyl and Si-based protecting groups..

The phosphorylation of the free hydroxyls of a suitably protected carbohydrate is usually performed using a P^{III} reagent. The resulting phosphite ester is immediately oxidised to give the protected phosphate ester. Examples of phosphoramidites used in the synthesis of carbohydrate analogues are given in figure 2.2. They are activated by a weak acid (1*H*-tetrazole). The oxidation of phosphite triesters is usually effected with *tert*-butyl hydroperoxide (*t*BuOOH) or *m*-chloroperoxybenzoic acid (*m*CPBA).

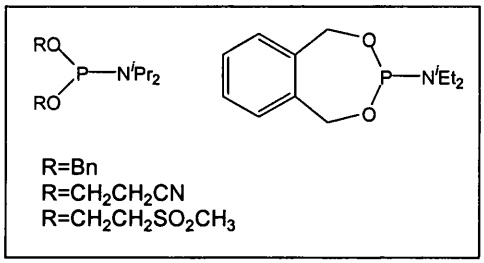


Figure 2.2: Phosphitylating reagents.

In order to avoid migration of protected phosphate esters to adjacent free hydroxyl groups it is essential to deblock the phosphate groups before the alcohols. Benzyl groups are convenient as benzyl phosphate esters are cleaved more rapidly than the benzyl ethers therefore deprotection in one step is possible. Benzyl groups are usually removed by hydrogenolysis. When base sensitive phosphitylating reagents are used, a two stage deprotection is usually required. The final compounds may be purified by anion-exchange chromatography or HPLC.

2.2 Minimal Structures

Adenophostin analogues that lacked the adenine base of the adenophostins or similar structure have been termed minimal structure analogues. These compounds can be split into two categories; those based on a single ring "monosaccharide" derivatives and those based on two rings "disaccharide" derivatives. As two of the chapters in this thesis are about minimal structure analogues the biological results and structure activity

relationships of the following compounds will be discussed in the introduction of chapters 2 and 3 to explain the rationale of the compounds described in the chapters.

2.2.1 Monosaccharide analogues

The first monosaccharide analogue prepared was (2-hydroxyethyl)-α-D-glucopyranoside-2',3,4-trisphosphate [Glc(2',3,4)P_3]. It is structurally based on the α-glucoside moiety of adenophostin A and can be visualised as adenophostin A with the majority of the adenosine moiety excised. Two groups independently reported the synthesis of this analogue almost simultaneously.

Jenkins and Potter[46] started the synthesis with the known allyl glucopyranoside, regioselective benzylation at positions 2 and 6 was achieved using tin stannylene methodology (Figure 2.3). Temporary acetal protection of the 2,3 triol with an isopropylidene group allowed oxidative cleavage of the double bond followed by reduction. The triol was isolated, phosphorylated and deblocked to afford the trisphosphate.

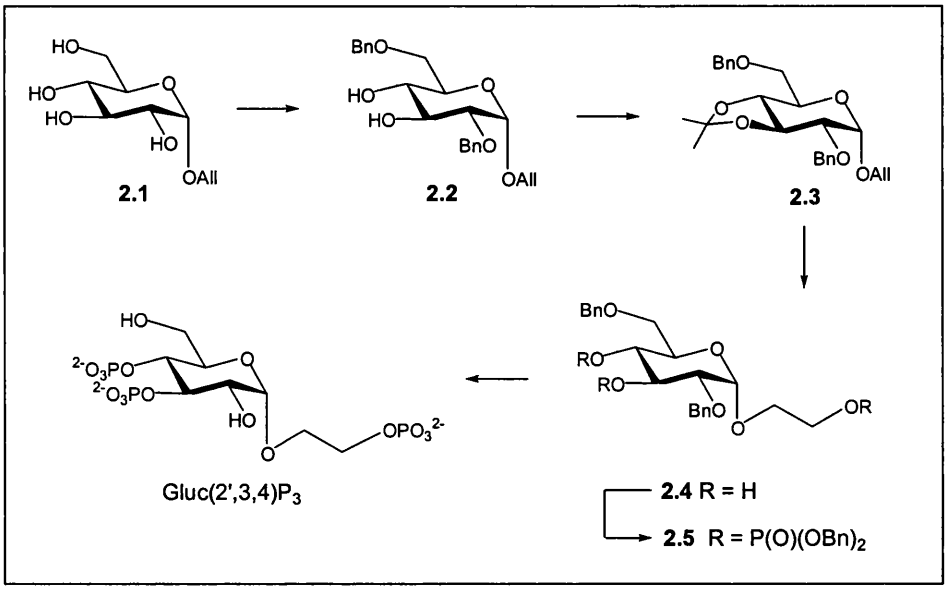


Figure 2.3: Route to Glc(2'3,4)P_3.

The other group Wilcox and Gigg[47;48] only communicated their synthesis; their extensive biology and structure activity relationships will be discussed in chapter 2.

At a similar time another series of compounds was reported by Moitessier *et al*[49]. These compounds were based on the sugar D-xylose and contained the same

hydroxyethyl side chain as the compound described above but with both α and β stereochemistry. They also synthesised the α and β compounds with propyl side chains. The synthesis was similar to that described above for Glc(2',3,4)P_3; thus after appropriate protection, ozonolysis of the double bond in the side chain, followed by reductive work up with sodium borohydride gave the precursors for the expected hydroxyethyl xylosides. The corresponding 3-hydroxypropyl derivatives were obtained by hydroboration of the double bond in **2.6**. The four triols were phosphitylated and oxidised to give the trisphosphate precursors, which were deprotected by hydrogenolysis.

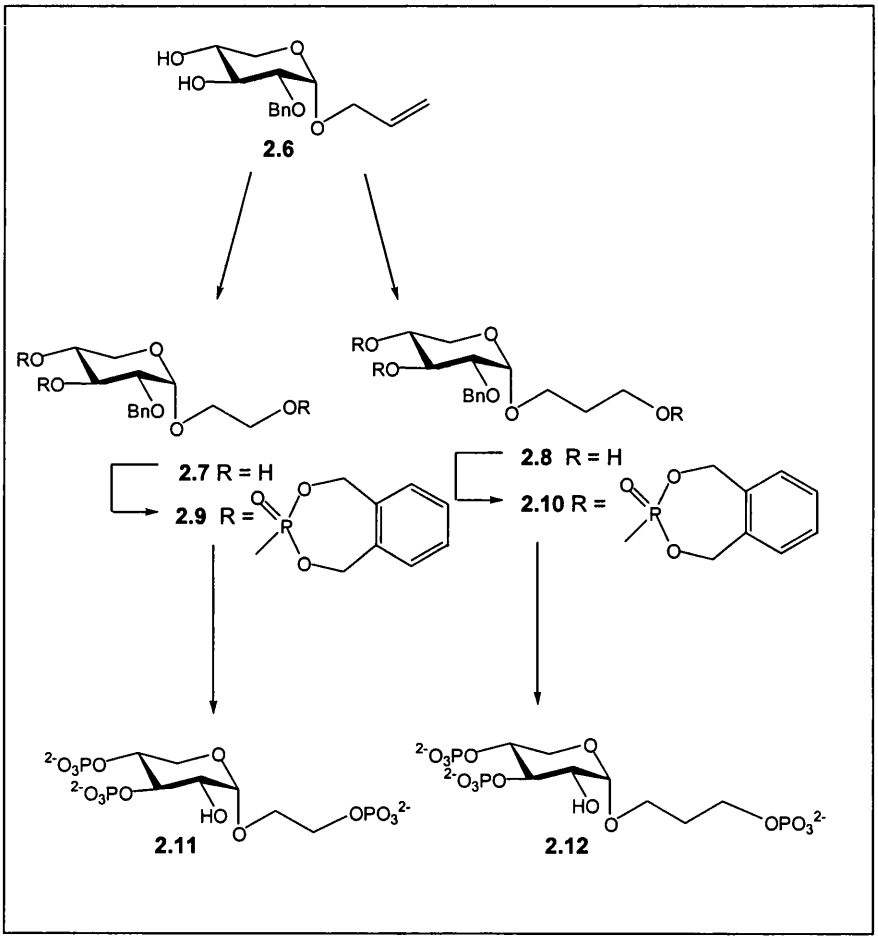
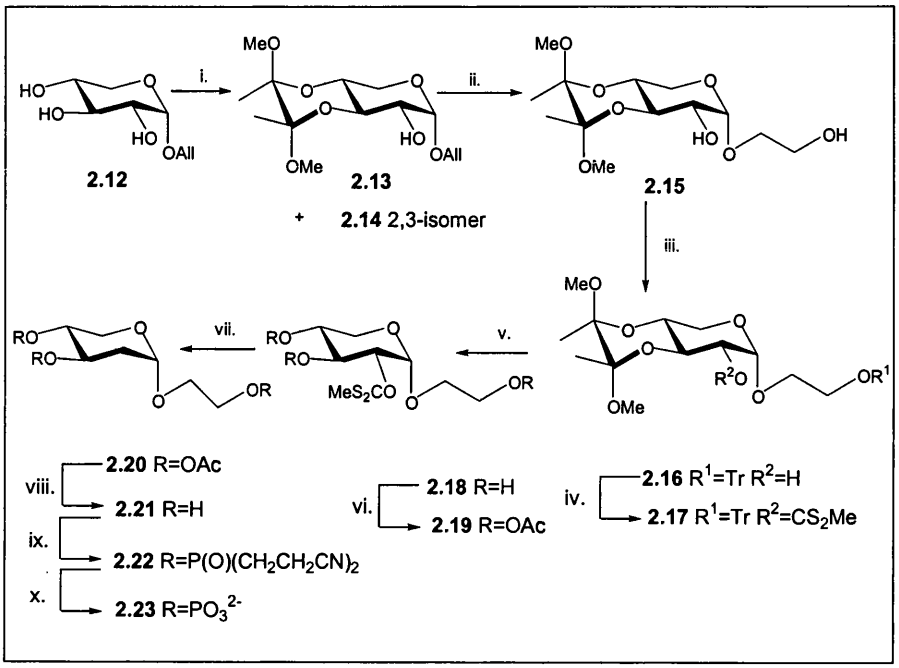


Figure 2.4: Route to the α-xylose analogues **2.11** and **2.12**.

A few years later the same group published the synthesis of the 2-deoxy analogue **2.23**[50]. This time, the allyl D-xyloside was submitted to acetal formation by reaction with butanedione in the presence of a catalytic amount of camphorsulfonic to give an equal amount of **2.13** and **2.14** which could be separated. The double bond in **2.13** was cleaved by ozonolysis to give, after reduction, the primary alcohol which was protected as the trityl derivative. The C-2 hyroxyl group was then converted into its methyl

xanthate, the protecting groups were removed and replaced with acetyls to give **2.19**, which was deoxygenated using the Barton-McCombie method. **2.20** Was deprotected to give triol **2.21**. Phosphitylation and oxidation gave the protected trisphosphate, which was deblocked to give the final compound.



Scheme 2.1: Synthetic route to 2-deoxy-α-D-*threo*-pentopyranoside.
Reagents and conditions: i) $MeC(OMe)_2C(OMe)_2Me$, CSA, MeOH, $(MeO)_3CH$, reflux 90 min; ii) O_3, CH_2Cl_2/MeOH, –70 °C, 90 min then $NaBH_4$; iii) TrCl, DMAP, pyridine, 80 °C, 30 h; iv) NaH, CS_2, THF then MeI; v) 95% aq. TFA–CH_2Cl_2 (1:1), rt, 10 min; vi) Ac_2O, pyridine; vii) Bu_3SnH, AIBN, toluene; viii) NaOMe, MeOH; ix) $(CNCH_2CH_2O)_2PN^iPr_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 4h then *t*BuOOH, O °C; KOH, MeOH, 40 °C, 2.5 h:

In Chapter 3, the synthesis and biological evaluation of α and β *C*-glycosides will be described and other *C*-glycoside Ins(1,4,5)P_3 analogues will be compared.

2.2.2 Conformationally restricted analogues

The first conformationally restricted analogue of Ins(1,4,5)P_3 was designed and synthesised in this laboratory[51]. The cyclic phosphate analogue in which the phosphate group equivalent to the 4-phosphate of Ins(1,4,5)P_3 was tethered *via* a methylene group to the equivalent carbon at position 3 in Ins(1,4,5)P_3. Unfortunately this compound was inactive but inspired the synthesis of carbohydrate based conformationally restricted analogues. The spirophostins[52] were designed to study in

detail the effect of the position of the 2' phosphate in adenophostin A. Chain elongation of the protected thioethylglucoside gave a mixture of diastereoisomers which was subjected to spiroketalization. Separation of the intermediate spiroketals, followed by phosphorylation and deprotection afforded the spirophostins (**2.27** and **2.28**).

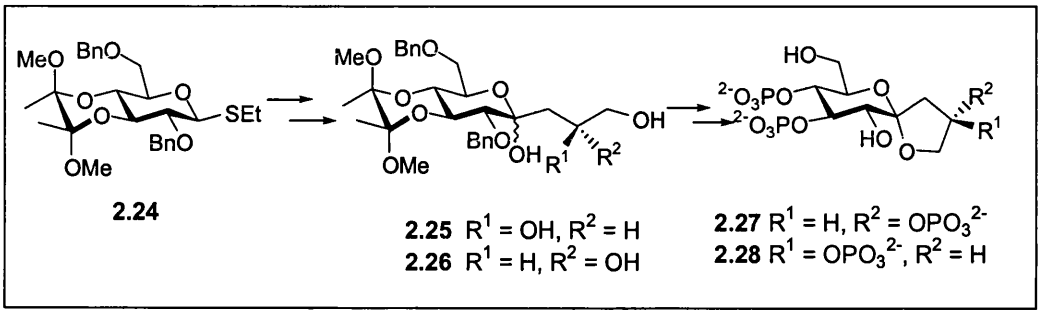


Figure 2.5: Synthesis of spirophostins.

Both spirophostins are approximately equipotent with respect to their Ins(1,4,5)P_3R binding and Ca^{2+} release properties. They are both less potent than Ins(1,4,5)P_3 and adenophostin A. At a similar time a report was published from this laboratory describing bicyclic[53] analogues of Ins(1,4,5)P_3 (Figure 2.6), these analogues were designed to place their non-vicinal phosphate groups in regions of space more distant from the inositol ring than are normally accessible to the 1-phosphate group of Ins(1,4,5)P_3. Interestingly even though the molecules constrain the non-vicinal phosphate group to a significantly different region of space, all the molecules have similar potencies.

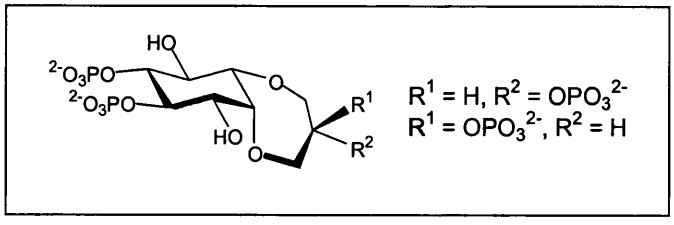


Figure 2.6: Bicyclic analogues based upon adenophostin A.

2.2.3 Disaccharide analogues

Disaccharides offer plenty of scope as frameworks for the synthesis of novel polyphosphates, but the difficulties of protecting the hydroxyl groups selectively are often much greater than those encountered with simple inositols. There are two main methodologies for synthesising disaccharide polyphosphates.

The first method is to choose an existing disaccharide with the required anomeric configuration, selectively protect some of its hydroxyl groups, phosphorylate the unprotected positions and then deprotect. However as many disaccharides are not available, precise target structures may not be accessible. Three compounds have been synthesised in this manner(Figure 2.7)[54].

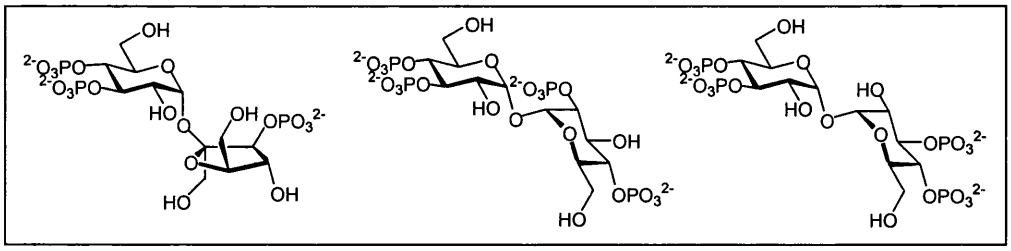
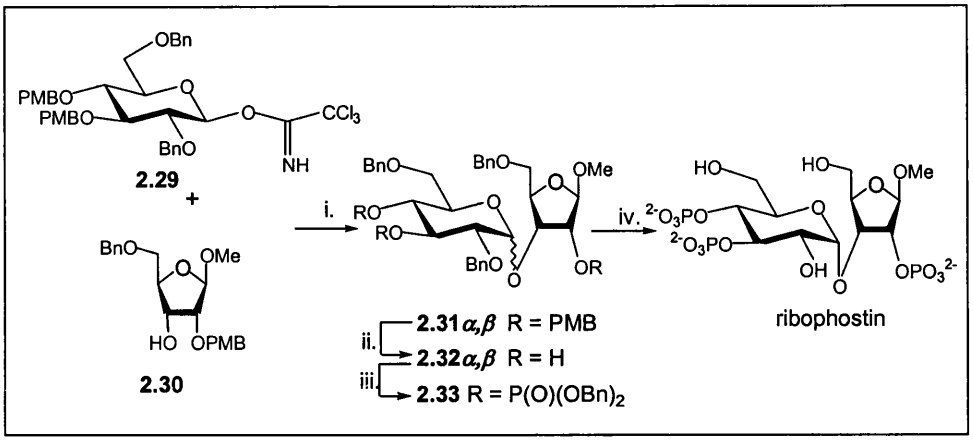


Figure 2.7: Disaaccharide polyphosphates

The second method is more adaptable; it relies on the selective protection of the hydroxyl groups in two monosaccharides and the coupling of the two together. This method has the advantage that any desired disaccharide polyphosphate can be assembled. A number of compounds have been synthesised in this manner.

The first disaccharide synthesised was called ribophostin (Scheme 2.2)[55]. It was prepared to mimic the structure of adenophostin A, but with the adenine removed. The glycosyl imidate was prepared in six steps from D-glucose using similar protecting group manipulations as described for Gluc(2',3,4)P_3. The methyl riboside was prepared in several steps from D-ribose by a series of protecting group manipulations to mask all the hydroxyl groups except the 3 position.

Coupling of **2.29** and **2.30** was achieved under standard Schmidt conditions using TMSOTf as promoter resulting in a 4:1 α:β mixture of anomers. After removing the PMB protecting groups the α anomer was isolated. **2.32**α was phosphorylated to **2.33**, deprotection by hydrogenolysis yielded the ribophostin.



Scheme 2.2.: Synthetic route to ribophostin.
Reagents and conditions: i) $Me_3SiOSO_2CF_3$, Et_2O, 3 Å sieves, rt, 10 min; ii) DDQ, CH_2Cl_2–H_2O (10:1), rt, 1 h, (56%); iii)$(BnO)_2PNPr^i_2$, 1*H*-tetrazole, rt, 30 min, then *M*CPBA, –78 °C to rt, 10 min, (82%); iv) H_2, Pd–C, 40 psi, 16 h, (70%):

The Matsuda group designed and synthesised a number of glycosyl-tetrahydrofuran trisphosphates. A fluoroglycoside donor was used to couple to a number of tetrahydrofuran derivatives (Figure 2.8)[56].

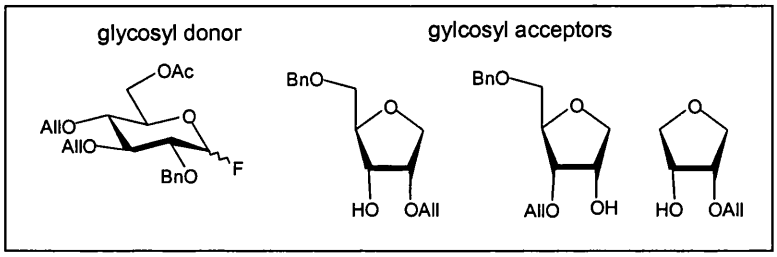


Figure 2.8: Glycosyl donor and acceptors

Glycosidation reactions of the fluoroglycoside donor with the different acceptors using TMSOTf as a catalyst gave the corresponding α-glycoside as the main product. After protecting group manipulations the corresponding triols were isolated. Phosphorylation was achieved by the classical phosphoramidite methodology to provide after deprotection the three corresponding trisphosphates (Figure 2.9).

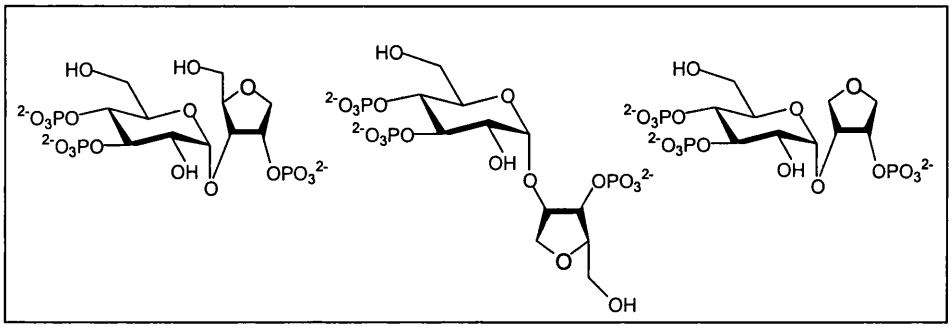


Figure 2.9: More disaccharide polyphosphates.

The Potter group also synthesised furanophostin [57] in a similar manner to the four disaccharide analogues described in chapter 4.

One interesting application of disaccharide polyphosphates was to construct clustered disaccharide polyphosphates[58;59]. These were designed to help study the Ins(1,4,5)P_3-binding events to the tetrameric receptor. The disaccharide (described in chapter 5) was elaborated to **2.34**. This was used in a coupling reaction with different iodobenzene derivatives to give the fully protected precursors. Deacetylation, phosphorylation and deprotection by hydrogenolysis yielded compounds **2.35–2.37**.

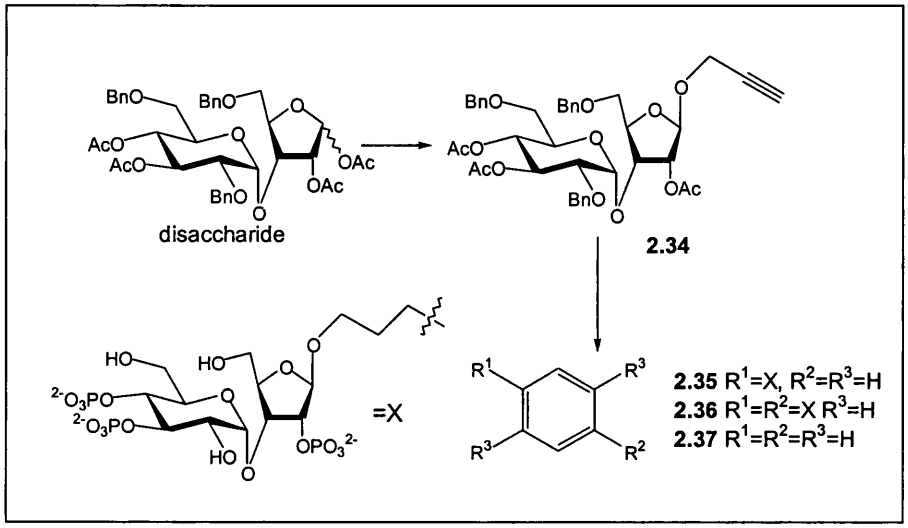


Figure 2.10: Synthesis of clustered disaccharide polyphosphates.

2.3 Base containing analogues

Several adenophostin analogues which retain the purine base have been synthesised. This section deals with the chemistry of these analogues, as well as some of their structure activity relationships.

2.3.1 Total synthesis of adenophostin A

Three total syntheses of adenophostin A have been reported so far. The first total synthesis was completed by the Sankyo group[42] whose aim was to provide definitive proof of the chemical structure of adenophostin A. Briefly, glycosylation of a regioselectively protected glucosyl bromide donor derivative with a regioselectively protected adenosine-based acceptor using silver perchlorate and γ-collidine as promoters gave the basic backbone in 48% yield (figure 2.11). The remainder of the synthesis consisted of protecting group manipulations to selectively protect the two primary

alcohol positions and the amino group of the adenine. Subsequent phosphorylation and removal of the protecting groups gave adenophostin A in eight steps from the two protected starting materials.

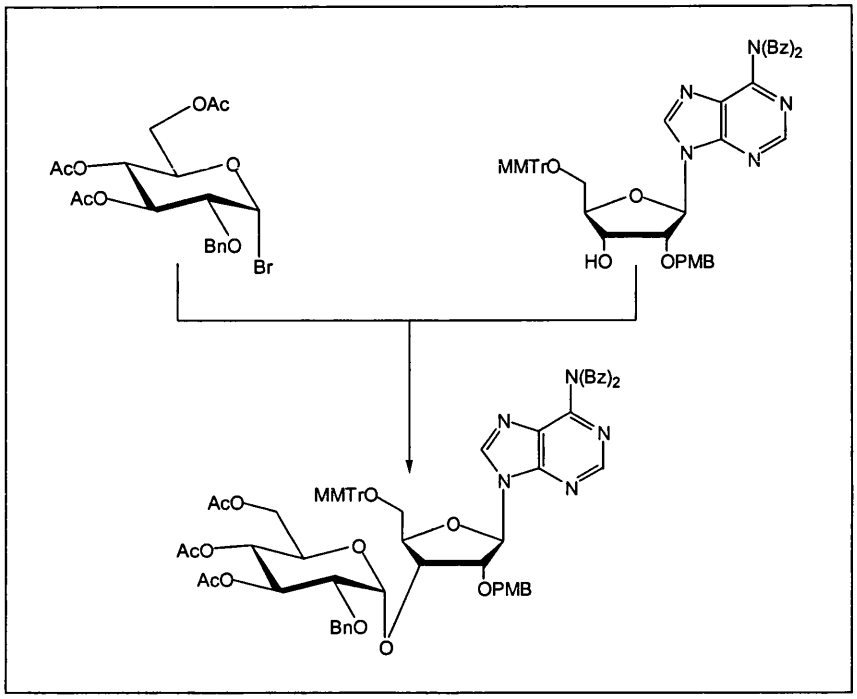


Figure 2.11: Glycosidation.

More recently our group[60] adopted a similar but more efficient strategy. This was based on glycosidation of a regioselectively protected adenosine-based acceptor with a regioselectively protected glycosyl phosphite donor. The α-coupled product was the sole product. Deprotection of the acid sensitive groups with TFA in dichloromethane led to the required triol. Selective phosphitylation, without amino group protection, was achieved using a stoichiometric amount of imidazolium triflate instead of tetrazole as the phosphoramidite activator. Subsequent oxidation and deprotection by catalytic hydrogenation gave adenophostin A. (See figure 2.15, strategy 1)

The third approach, used by van Straten *et al*[61], involved a very different convergent approach whereby the adenine base was introduced onto a disaccharide intermediate using Vorbrüggen condensation methodology. This kind of approach will be discussed in more detail in chapter 5.

2.3.2 Molecular Modelling of adenophostin A

Early modelling studies by Takahashi *et al* [44] and Wilcox *et al* [48] indicated that the vicinal equatorial 4"- and 3"-bisphosphates and the α-glucoside ring of adenophostin A can be superimposed on the vicinal equatorial 4- and 5-bisphosphates of the *myo*-inositol ring of Ins(1,4,5)P_3. The modelling demonstrated that the 2'-phosphoryl group of adenophostin A relative to the 3" and 4"-bisphosphate motif might be slightly more extended than the corresponding positions of Ins(1,4,5)P_3, suggesting that the high potency of adenophostin might be due to the optimal positioning of the 2'-phosphoryl group.

Later Hotoda *et al*[62] suggested a putative conformation of adenophostin A at the Ins(1,4,5)P_3R. Their suggestion is based upon molecular mechanics calculations and NMR experiments in solution. They found the following;

- The glucose moiety adopts a chair conformation with two equatorial phosphoryl groups;
- The ribose ring prefers the south (C2'-*endo*) conformation rather than north (C3'-*endo*);
- The N-glycosyl linkage was found to be in the *syn* orientation, which may be stabilised by an O5'-N3 intramolecular hydrogen bond.

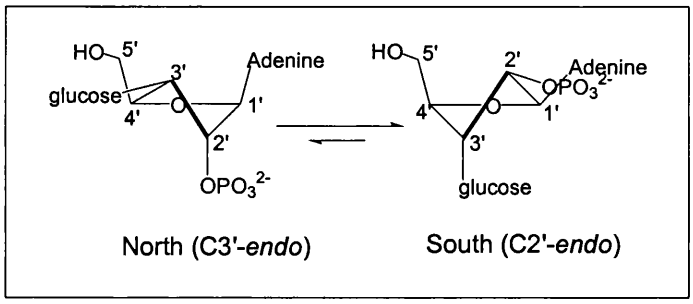


Figure 2.12: North and south ribose conformations.

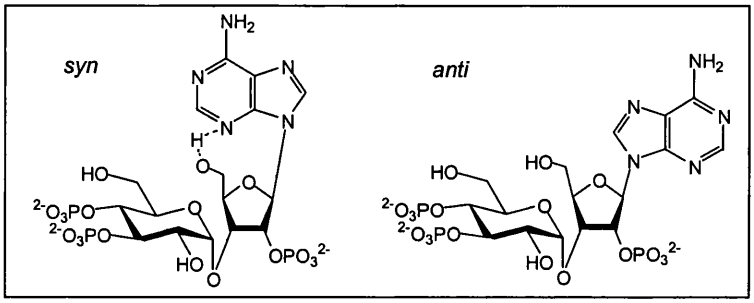


Figure 2.13: *Syn* and *anti* conformations of adenophostin A.

They proposed that their model indicates the possibility that the relative position of the 2'-phosphate group of adenophostin A is optimal for the recognition by the Ins(1,4,5)P_3R.

The model[48] is inconsistent with the Hotoda model[62]. The Wilcox conformation was compactly folded and the N-glycosyl linkage was *anti*. This conformation is inconsistent with Hotoda's NOE data observed between H-8 and H-1' and H-8 and H-2'. Furthermore, when Hotoda modelled the Wilcox conformer it was found to be less stable than their model. Finally the Hotoda model is consistent with our model, based on molecular dynamics simulation of adenophostin A.[60].

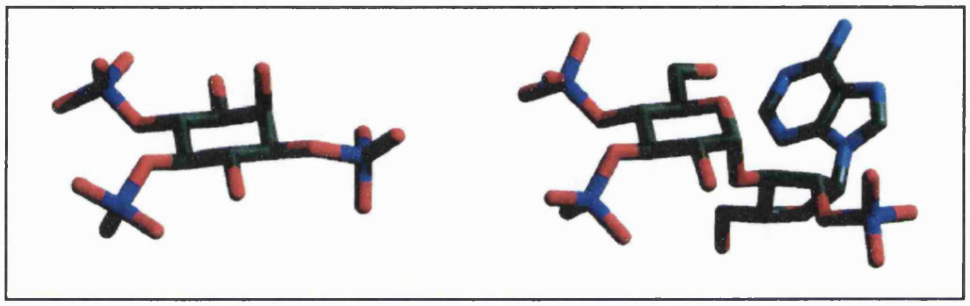


Figure 2.14: Comparison of conformations of X and Y. Left: structure of Ins(1,4,5)P_3 taken from X-ray crystal structure of the phospholipase C-δ1 pleckstrin homology domain complex with Ins(1,4,5)P_3. Right: representative energy-minimised conformer of adenophostin A obtained from molecular dynamics simulations at 300 K.[60]

2.3.2 Adenophostin A analogues by total synthesis.

The two distinct types of approaches to the synthesis of adenophostin A have led to the synthesis of two main classes of analogues. Substitution of the glycosyl donor in the first methodology by a different sugar-derived donor has led to two congeners modified at glucose. While replacing the adenine base in the second strategy has led to a whole series of nucleobase-modified adenophostin A mimics. The two strategies are summarised in figure 2.15.

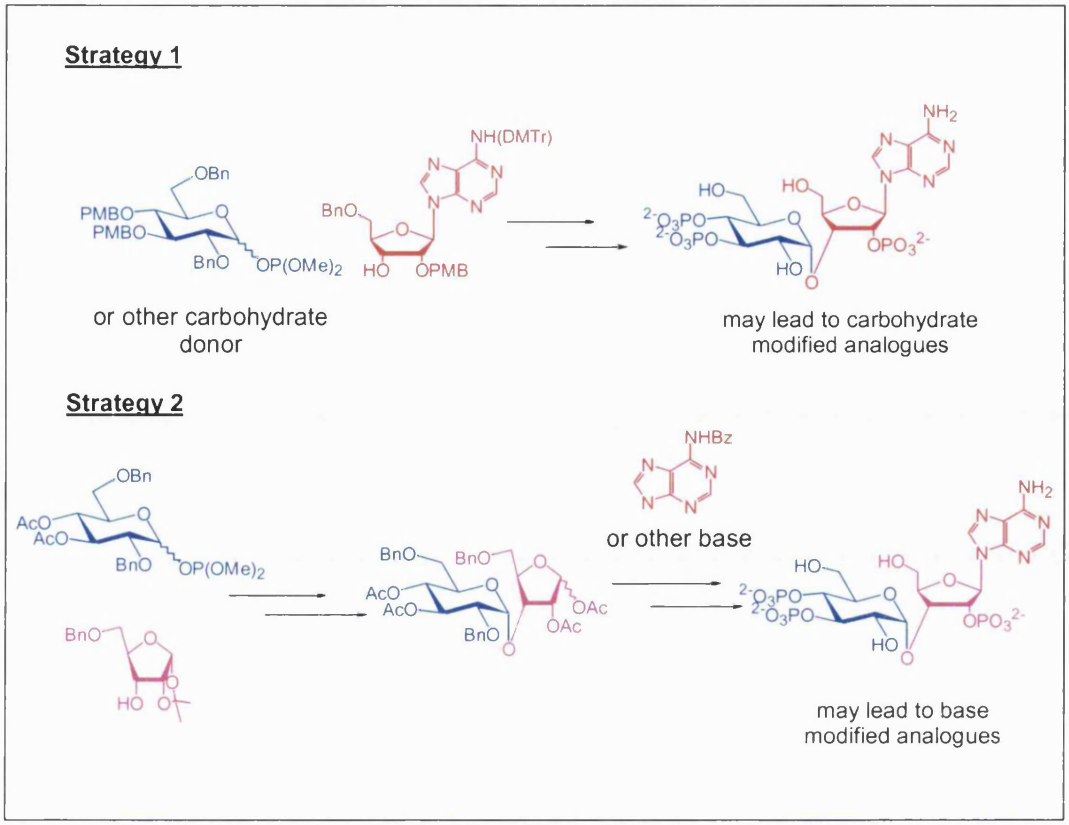


Figure 2.15: The two strategies for the synthesis of adenophostin A and analogues.

2.3.4 Carbohydrate modified analogues

The glucopyranosyl bisphosphate moiety was replaced with mannopyranosyl bisphosphate and xylopyranosyl bisphosphate units respectively[63]. Evaluation of the analogues showed *manno*-adenophostin to be about twelve fold less potent than adenophostin while *xylo*-adenophostin is only two fold less potent than adenophostin[43].

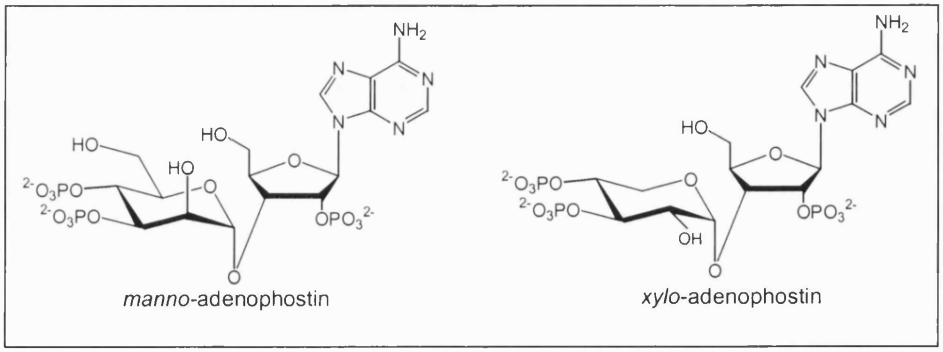


Figure 2.16: Strucures of *manno*-adenophostin and *xylo*-adenophostin.

2.3.5 Base modified analogues

Many base-replaced analogues have been synthesised[64;65]. We will take a closer look at these compounds in Chapter 4.

2.3.6 Flexible analogues

Several analogues in which the ribose or the glucose have been cut up in different ways have been reported (Figure 2.17).

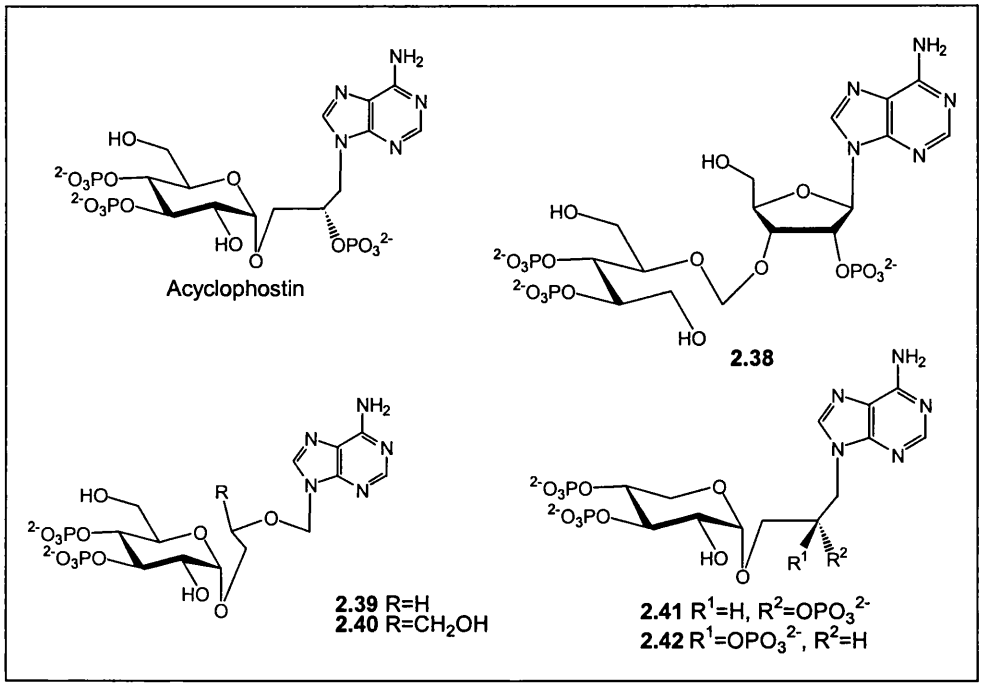
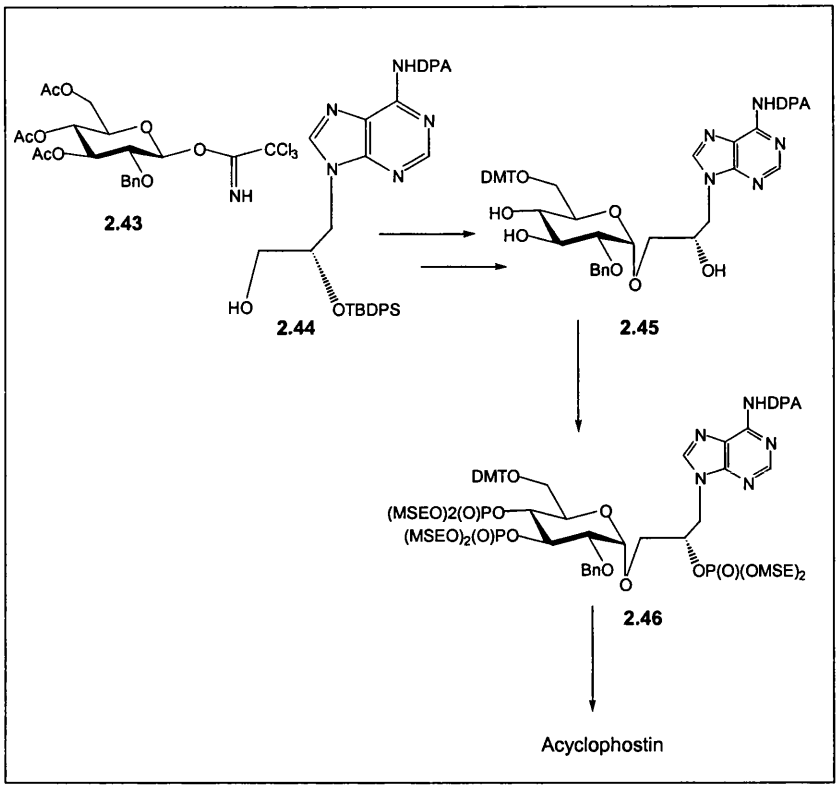


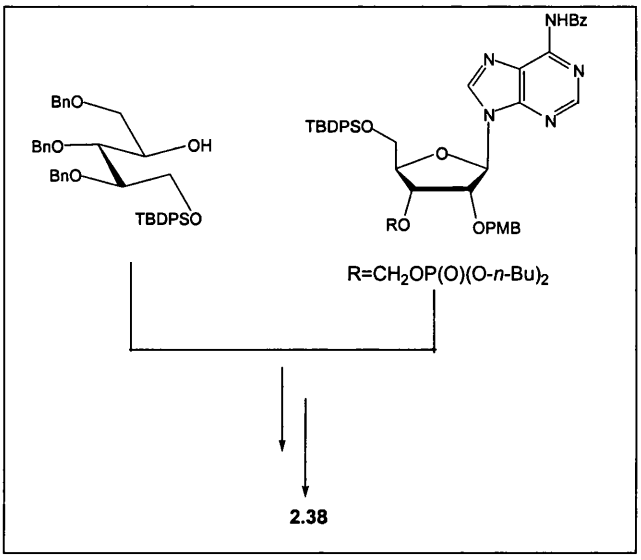
Figure 2.17: Flexible analogues of adenophostin A.

The synthesis of acyclophostin[66] commenced with the condensation of **2.44** with the glucopyranosyl trichloroacetimidate donor (**2.43**). The glycosidation under controlled addition of excess TMSOTf proceeded smoothly to give the α-compound exclusively (**2.45**). Protecting group manipulations gave the corresponding triol which was phosphitylated with *N,N*-diisopropyl-bis-[2-methylsulfonyl]ethyl phosphoramidite (MSE). It was subsequently oxidised with *tert*-butyl hydroperoxide to give the fully protected trisphosphate. Removal of the base labile groups in **2.46** with NaOH, followed by aqueous acid mediated removal of the DMT-group and finally hydrogenolysis led to the target compound.



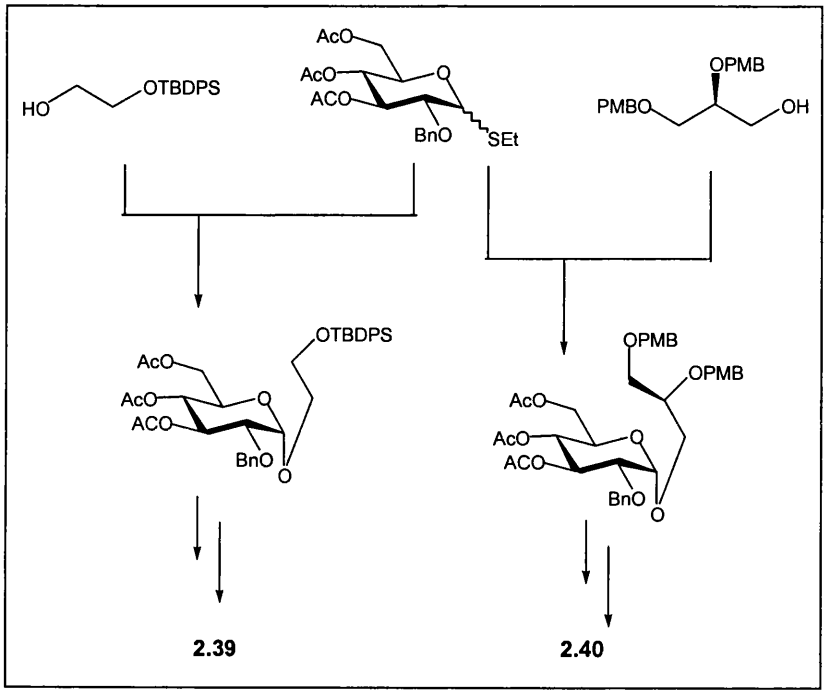
Scheme 2.18: Synthesis of acyclophostin.

A second compound **2.38** which lacked the C-1"–C-2" was prepared in the same report. Condensation of the adenosine donor with arabinitol acceptor under the influence of TMSOTf led to the isolation of the dimer. Protecting group manipulations gave the triol which was subjected to phosphorylation and deprotected as described for acyclophostin.



Scheme 2.19: Glycosidation.

The same group then reported the synthesis of two more analogues of adenophostin A in which the ribosyl-2-phosphate unit is replaced by either a glycol or a glycerol[67]. Both syntheses took the same route; briefly, glycosidation of the glycosyl donor with the respective glycol and glycerol acceptors led to the α-linked compounds. The adenine was then introduced by Vorbrüggen condensation with the bis-trimethylsilyl derivative of N6-benzoyl adenine in the presence of a catalytic amount of TMSOTf. The resulting compounds were then submitted to routine protecting group manipulations to install the trisphosphate groups of the final compounds.



Scheme 2.20: Route to **2.39** and **2.40**.

The biological results of these compounds and a couple of related compounds were published in a subsequent paper[68]. Replacement of the glucose moiety by a bisphosphorylated polyol, as in the acyclic analogue had a detrimental effect. The opening of the ring presumably allows too much conformational mobility around the essential bisphosphate and neighbouring hydroxyl group.

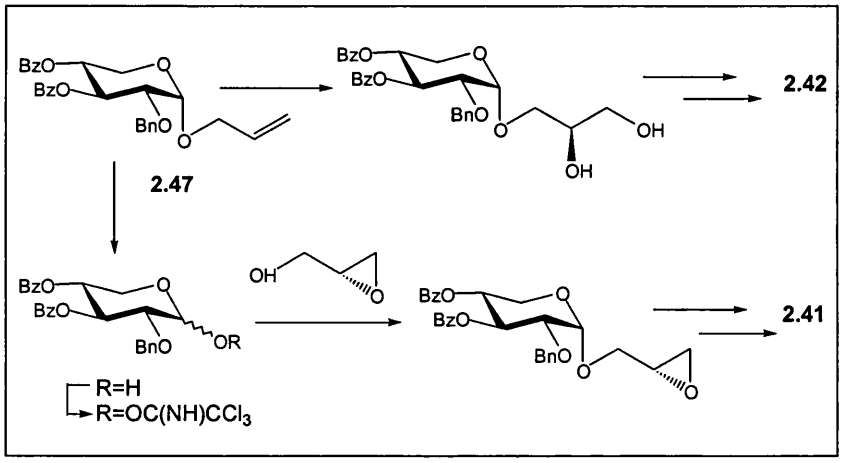
The substantial decrease in the affinity of the bisphosphate analogue **2.39**, **2.40** parallels the massive decrease in affinity of adenophostin after the removal of its 2' phosphate[41].

Interestingly, acyclophostin exhibited unusual activity. It bound to hepatic membranes with appreciably higher affinity than Ins(1,4,5)P_3, yet it was marginally less potent in causing Ca^{2+} mobilisation. Further investigation demonstrated that

acyclophostin was in fact a pH dependent partial agonist; it was found to be a partial agonist at high pH (8.3) and a full agonist at pH 7. It was speculated that the pH dependent efficacy of acyclophostin was either the result of a conformational change related to the pKa of the 2'-phosphate and the lack of conformational restriction from a furanoside ring, or that acyclophostin may differ from $Ins(1,4,5)P_3$ in the residues involved in its binding and Ca^{2+} release at the $Ins(1,4,5)P_3$ receptor.

The Chapleur group recently reported the synthesis of the acyclophostin related compounds **2.41** and **2.42**[69]. The two compounds differ only by the stereochemistry at the C-2 of the propyl tether between the xylose ring and the adenine.

Asymmetric dihydroxylation of the dibenzoate gave the diol in an 8:1 mixture of diastereoisomers. Activation of the primary alcohol as the tosylate and the reaction with the sodium anion of adenine led to a mixture of N-7 and N-9 regioisomers. The N-9 isomer was isolated, and the benzoates removed to give the triol. The resulting triol was phosphitylated followed by *in situ* oxidation of the corresponding phosphites gave the protected phosphate derivative. Removal of all the benzyl protecting groups gave the required compound.



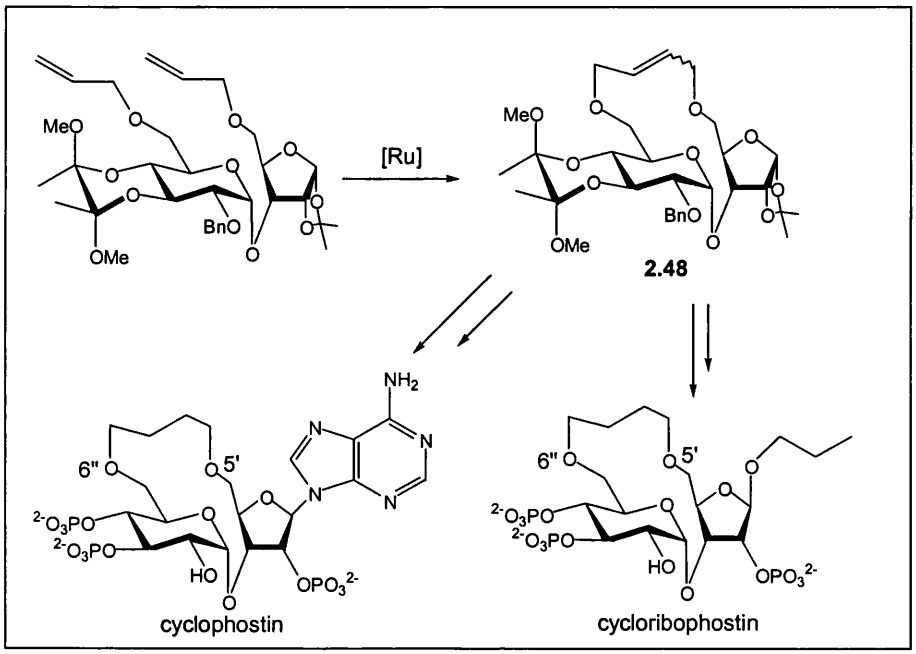
Scheme 2.21: Routes to compounds **2.41** and **2.42**.

The second isomer was synthesised via a different route. It commenced with the deallylation of compound **2.47** to provide the free hemiacetal. Activation of the free hemiacetalic hydroxyl was acheived via the Schmidt procedure. The imidates were then reacted with enantiomerically pure (R)(+) glycidol using TMSOTf as promotor to give a mixture of anomeric glycosides. The α-anomer was isolated and the epoxide ring was opened with the adenine anion. The remainder of the synthesis was identical to that described above for compound **2.42**.

These compounds were tested for Ca^{2+} release from permeabilised hepatocytes. Compound **2.41** was about five times weaker at releasing Ca^{2+} than Ins(1,4,5P_3 while compound **2.42** was 10 times weaker. The significance of the biological results of these two compounds will be discussed in chapter 3.

2.3.7 Conformationally restricted analogues

A paper recently appeared in which the authors described the synthesis and biological evaluation of a 5',6" tethered analogue of adenophostin A called cyclophostin, as well as its de-adeninylated analogue cycloribophostin [70]. The compounds were prepared via ring closing metathesis of a carbohydrate dialkene, followed by coupling of **2.48** to 6-N-benzoyl adenine or propargyl alcohol, respectively. The compounds were deprotected to give triols. Deprotection after phosphorylation gave the final compounds.



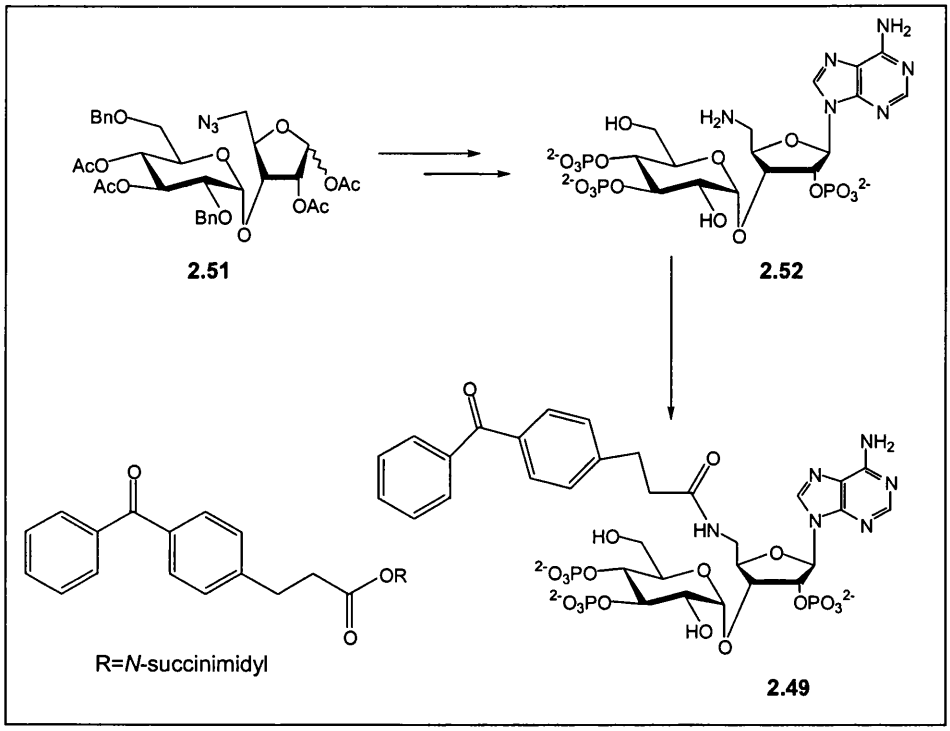
Scheme 2.22: Ring closing metathesis-approach towards cyclophostin and cycloribophostin.

Cyclophostin is approximately five fold more potent at releasing Ca^{2+} than Ins(1,4,5)P_3. In contrast, the potency of cycloribophostin is greatly reduced, confirming that the adenine is important in achieving adenophostin like activity. NMR spectroscopy and molecular modelling of these analogues showed that the 5',6" tether induces a C-3'-*endo/anti* conformation, rather than C-2' *endo/syn* conformation of adenophostin.

2.3.8 Photoaffinity derivatives

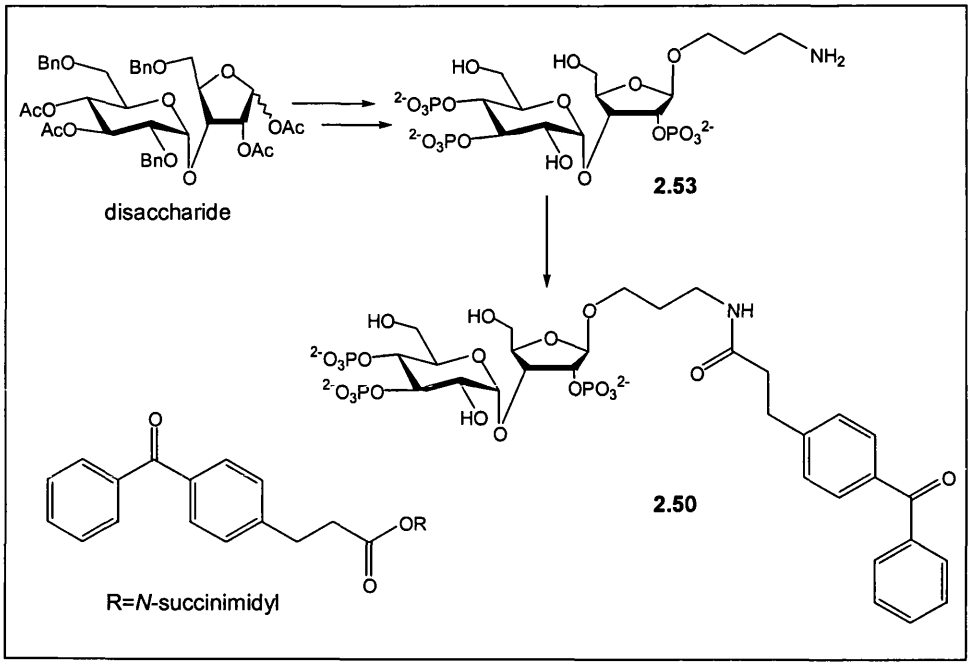
Two photoaffinity derivatives have recently been reported[71]. In compound **2.49**, the chemically stable *p*-benzoyldihydrocinnamoyl (BZDC) photoprobe is joined directly by an amide bond to the 5'-position, while in **2.50** it is tethered to an aminopropyl spacer at the anomeric centre of the ribosyl moiety.

The usual Vorbrüggen type condensation of **2.51** with N-6 benzoyl adenine gave the backbone. The standard protecting group manipulations were applied, followed by phosphorylation and deblocking yielded **2.49**. Condensation of the amino derivative **2.52** with *p*-benzoyldihydrocinnamoyl (BZDC) photoprobe proceeded in a chemoselective fashion to give **2.50**.



Scheme 2.23: Route to photoaffinity compound **2.49.**

Glycosidation of the disaccharide with 3-(benzyloxycarbonylamino)-1-propanol under the influence of $SnCl_4$ led to the exclusive formation of the β-aminopropyl derivative. Usual deacetylation and subsequent phosphorylation gave the trisphosphate in 77% yield. Hydrogenolysis afforded derivative **2.53** which underwent a condensation reaction with BZDC photoprobe to yield **2.50**.



Scheme 2.24: Route to photoaffinity compound **2.50**.

Chapter Three

C-Glycosides as monosaccharide analogues

Chapter 3

3 C-Glycosides as monosaccharide analogues

3.1 Introduction

Adenophostin A can be viewed as a phosphorylated glucose glycosidically linked at its 1"-position to an adenosine at the 3'-position. As previously mentioned in the introduction, adenophostin analogues that lack the adenine base or a similar structure were termed minimal structure analogues, and these could be split into monosaccharides and disaccharides. The minimal structure analogues were designed to show the functional consequences of systematically trimming the adenosine. All were based on carbohydrates with the exception of 6-deoxy-6-hydroxymethyl-*scyllo*-inositol 1,2,4-trisphosphate (**3.1**) which was based on *scyllo*-inositol (**3.2**)[72].

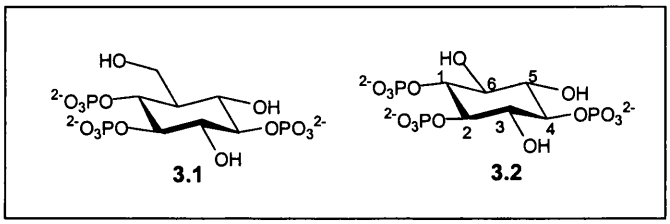


Figure 3.1: 6-deoxy-6-hydroxymethyl-*scyllo*-Ins(1,2,4)P_3 and *scyllo*-Ins(1,2,4)P_3

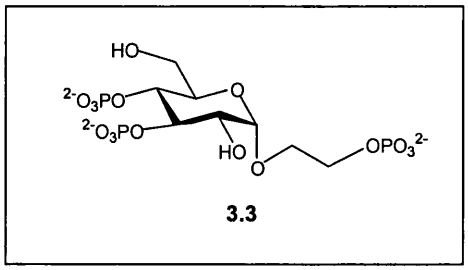
3.1 May be considered as an inositol based analogue of the adenophostin A glucose motif, with the 4,5-bisphosphate of Ins(1,4,5)P_3 being equivalent to the 3",4"-bisphosphate of adenophostin A. Therefore, the position of Ins(1,4,5)P_3 most likely to correspond to the bulky 5"-hydroxymethyl group of adenophostin A is the 3-hydroxyl of Ins(1,4,5)P_3. **3.1** Was designed to explore the effect of introducing such a group into an Ins(1,4,5)P_3 structure.

Biological evaluation of racemic **3.1** in permeabilised rabbit platelets found that it exhibited equal potency in Ca^{2+} release to Ins(1,4,5)P_3, and in binding assays in rat cerebellar membranes **3.1** was found to be equipotent to Ins(1,4,5)P_3. This observation implies that the CH_2OH component is tolerated by the Ins(1,4,5)P_3R despite the additional steric bulk.

It must be noted that **3.1** differs from Ins(1,4,5)P_3 in two ways; it is not only modified at the 3-hydroxyl position but the 2-position is equatorial as opposed to axial. Therefore it was also tested against *scyllo*-Ins(1,2,4)P_3 and was found to be significantly more potent at releasing Ca^{2+} from rabbit platelets and in binding. We

can conclude, at least in *scyllo*-analogues of Ins(1,4,5)P_3, that replacement of the secondary hydroxyl group at the 3 position (6 position in *scyllo*-inositol) with an hydroxymethyl group enhances affinity for the Ins(1,4,5)P_3R.

As mentioned in chapter 2, the first carbohydrate analogue synthesised was Gluc(2'3,4)P_3, (**3.3**)[46;47]. **3.3** Could be visualised as a truncated version of adenophostin A where the 2' and 3' carbons of the ribose ring and their terminal phosphate group are retained but the remainder of the adenosine residue is excised. It was found to be a full agonist at the Ins(1,4,5)P_3R of rabbit platelets, but ten-fold weaker than Ins(1,4,5)P_3.



3.3

Figure 3.2: [Gluc(2'3,4)P_3].

A comprehensive biological study of **3.3** was carried out by another group[48]. It was found to be a full agonist for Ca^{2+} release in SH-SY5Y neuroblastoma cells and MDCK cells, being only 10–12 fold weaker than Ins(1,4,5)P_3. Furthermore, in binding assays of pig cerebellum **3.3** had only 5-fold lower affinity compared with Ins(1,4,5)P_3. Like adenophostin A, **3.3** was shown to be unaffected by both 3-kinase and 5 phosphatase enzymes. Molecular modelling indicated that the vicinal equatorial 4 and 3-phosphates and the α-glucoside ring of **3.3** can be almost perfectly superimposed on the analogous structural moieties of both adenophostin A and Ins(1,4,5)P_3. A molecular dynamics simulation was used to explore the position of the 2'-phosphate group in adenophostin A and the 2'-phosphate in **3.3**, which confirmed that in **3.3** the conformationally flexible hydroxyethyl phosphate was likely to adopt an extended conformation therefore preventing the 2'-phosphate from accurately mimicking either the 1-phosphate of Ins(1,4,5)P_3 or the 2'-phosphate of adenophostin A.

Shortly afterwards, another group published the synthesis of more analogues similar to **3.3** but based on xylopyranosides (figure 3.3)[49]. It was found that three of the mimics showed comparable activity to **3.3** releasing approximately the same amount of intracellular Ca^{2+} from permeabilised hepatocytes. However the potency

of these analogues was ten fold lower than that of Ins(1,4,5)P_3, with the larger and more flexible β-hydroxypropyl mimic having a much lower potency. Biological evaluation of **3.3** and the xylopyranosides in the same assay would be interesting to determine the contribution of the hydroxymethyl group to the activity. This point has been considered in more recent studies and will be discussed further in chapter 3.

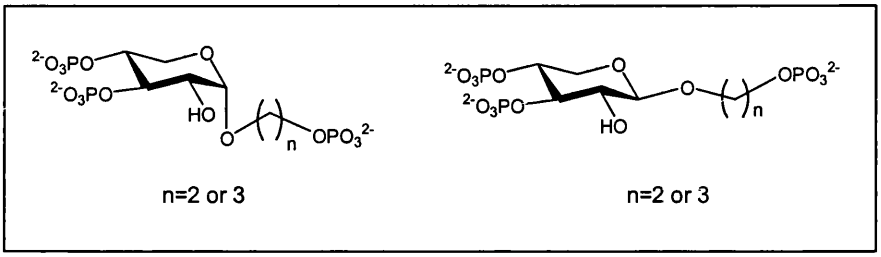


Figure 3.3: Xylopyranoside based minimal structures.

Later a report appeared on a synthesis of 2-deoxy derivative of Gluc(2'3,4)P_3[50]. Not surprisingly, this molecule was some 2,000-fold less potent than Ins(1,4,5)P_3 in mobilising intracellular Ca^{2+}, showing the expected parallel with the deletion of the 6-hydroxyl group of Ins(1,4,5)P_3.

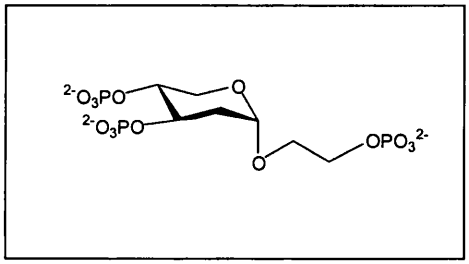


Figure 3.4: 2-deoxy derivative of Gluc(2'3,4)P_3.

3.2 Synthesis of the *C*-glycosides

The lower affinities of compounds (figures 3.2–3.4) compared with Ins(1,4,5)P_3 were thought to be due to the conformational flexibility of the side chain preventing the third phosphate from achieving an optimal binding position on the receptor. We therefore envisaged that it would be of interest to design a monosaccharide polyphosphate Ins(1,4,5)P_3 mimic, having the third phosphate closer to the ring and using a less flexible chain. It is clearly impractical to attempt synthesis of the chain–shortened version of Gluc(2'3,4)P_3, therefore a *C*-glycoside derivative was an attractive target. On the basis of all the above considerations, in the present work, we designed the syntheses of α and β-*C*-(hydroxymethyl)-1-deoxy-D-glucopyranoside 3,4, 1'-trisphosphate analogues (**1** and **2**) based on Gluc(2'3,4)P_3 but with a shorter side chain. In **1** and **2**, the third phosphate is attached to a carbon centre fixed in the α and β positions.

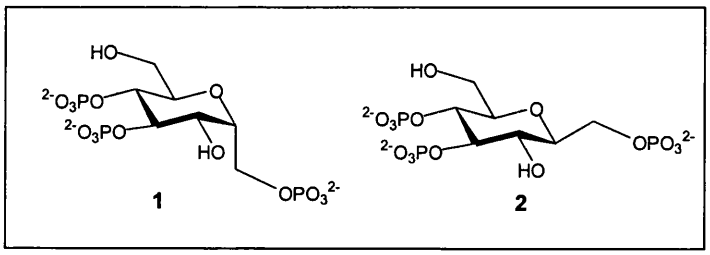


Figure 3.5: α and β-*C*-(hydroxymethyl)-1-deoxy-D-glucopyranoside 3,4, 1'-trisphosphate analogues

3.2.1 Synthesis of D-glucopyranose intermediate

The glucopyranose intermediate was produced by Fischer glycosidation with a slight modification of the method previously reported[46]. Thus D-glucose was reacted with allyl alcohol in the presence of HCl, which was generated *in situ* by careful addition of acetyl chloride, to give a mixture of pyranosides from which the α anomer was isolated in 31% yield. Regioselectively protected 2,6-dibenzyl-D-glucopyranose has already been prepared and reported in our laboratory[46]; however another method was sought, as this method involved stannylene mediated direct dibenzylation of positions 2 and 6 in **3**. Although this methodology was fairly successful on a small scale (44% on 2 g), when scaled up the yield reduced dramatically (15% on 35 g). Moreover, stannylene mediated dibenzylation requires extensive purification of the product by flash chromatography therefore limiting the

scale of the synthesis. This led to the investigation of an alternative route. Although the proposed route was longer it was hoped that the overall yield would be higher and require less time-consuming purification by chromatography.

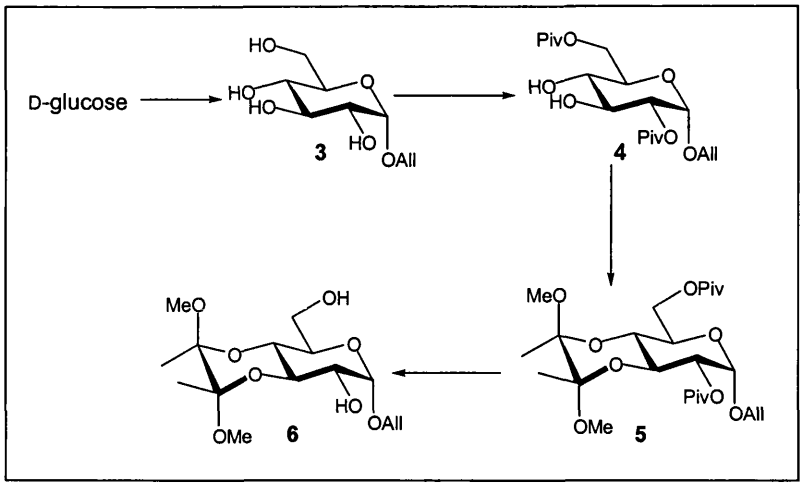


Figure 3.6: Route to the BDA protected derivative **6**.

A modification of a selective pivaloylation method described in the literature[73] was used to selectively acylate the 2 and 6-hydroxyls of **3**. This particular report was concerned with regioselective acylation of a selection of carbohydrates and treatment of methyl *α-D*-glucopyranoside was shown to give the 2,6-di-*O*-pivaloyl derivative in high yield (83%). Treatment of **3** with pivaloyl chloride (trimethylacetylchloride) at 0°C gave one major product which was shown to be the 2,6-di-*O*-pivaloyl derivative from the ^{1}H-^{1}H COSY NMR spectrum (which indicated typically deshielded doublet of doublets at δ_H 4.61 with coupling constants *J* 3.9 Hz and 9.8 Hz, corresponding to H-2, δ_H 4.42 and 4.33 with coupling constants *J* 1.9 Hz, 4.9 Hz and 12.2 Hz, corresponding to H-6).

The protection of the remaining *trans*-diequatorial hydroxyl groups at positions 3 and 4 was initially achieved by heating **4** under reflux with butane-2,3-dione, catalytic camphorsulphonic acid and excess trimethyl orthoformate for 3 h. This gave one major product (**5**), which, after purification, was identified as the corresponding butane diacetal (BDA) in 62.5% yield. The family of 1,2-diacetals (eg. BDA) have recently become very important protecting groups in carbohydrate chemistry and are extensively reviewed in [74]. Unfortunately, upon scale-up the yield of the reaction reduced dramatically due to the migration of the pivaloyl groups under acidic conditions. After the pivaloyl groups were removed using NaOH, the overall yield of the required product was poor (28%) over two steps.

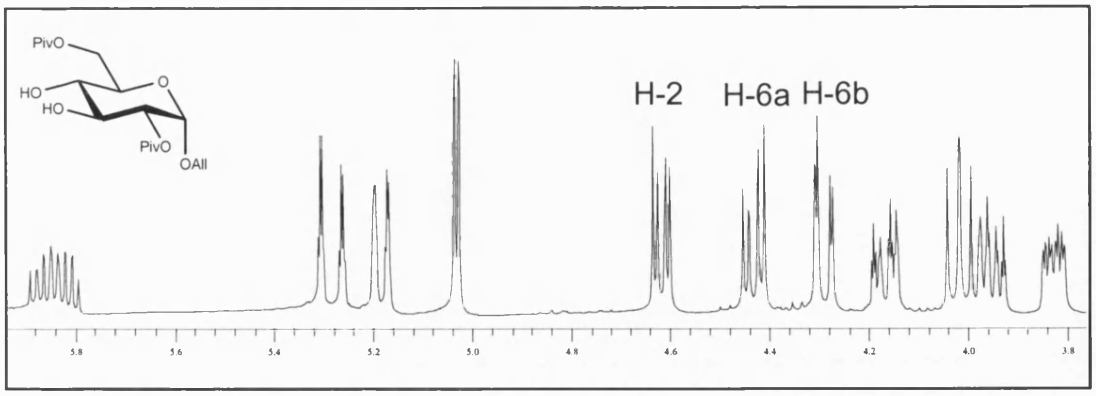


Figure 3.7: Part of the 1H NMR spectrum of **4** indicating the deshielded acylated positions of H-2 and H-6.

A slightly different strategy was adopted in which positions 3 and 4 of **4** were protected by an isopropylidene acetal using 2-methoxypropene in THF in the presence of a catalytic amount of *p*-toluenesulphonic acid. When **4** was used crude, a second product was formed during this reaction, presumably from some 2,3-di-*O*-pivaloyl ester formed in the preceeding step. Athough the impurity was not fully characterised, ^{13}C NMR identified this product as allyl 2,3-dipivaloyl-4,6-*O*-isopropylidene-α-D-glucopyranoside on the basis of characteristic δ_C values of isopropylidene quaternary carbons (δ_C 101.92, characteristic of a 6-membered ring) while **7** (δ_C 111.32, characteristic of a 5-membered ring)[75].

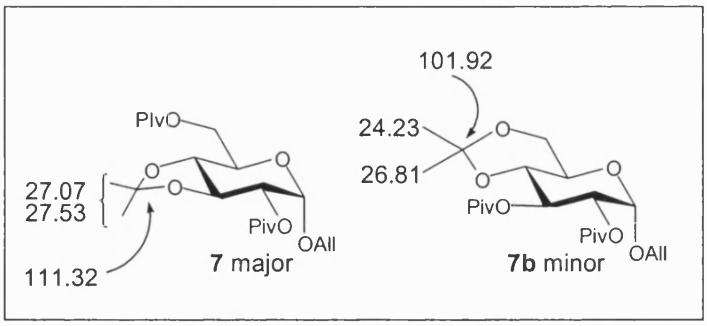
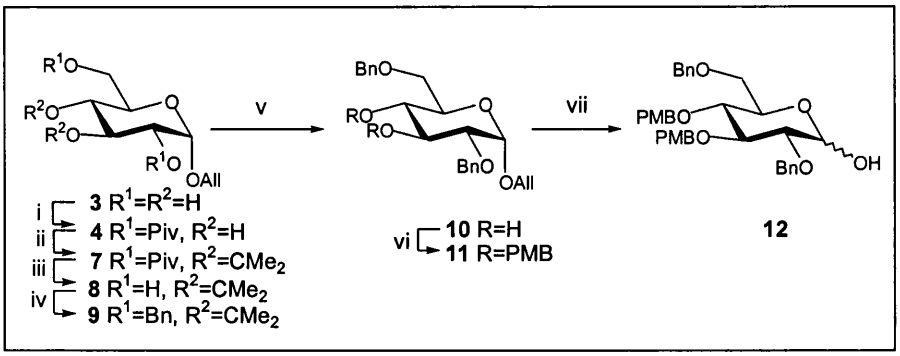


Figure 3.8: 400 MHz ^{13}C NMR chemical shift values for isopropylidene Cs in **7** and **7b**.

Conversion of **7** into the known allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside was then straightforward. The 2,6-di-*O*-pivaloyl derivative **7,** was saponified by heating at reflux with NaOH pellets in methanol. The resulting free 2- and 6-hydroxyls were alkylated using benzyl bromide and sodium hydride in DMF; the

product of this reaction was used without purification. Finally, the isopropylidene group was removed by stirring the crude intermediate with 10% 1M aqueous HCl in methanol for 30 min, and crystallisation from ether–hexane gave pure **10** (67% yield over five steps). This was found to be the most convenient and efficient method to produce **10** in multigram quantities. Similar reactions on D-xylose to produce selective allyl 2-*O*-benzyl-α-D-xylopyranoside (chapter 4), confirmed the general applicability of this method.



Scheme 3.1: Synthetic route to the D-glucopyranose intermediate.
Reagents and conditions: i)$(CH_3)_3CCOCl$, pyridine, 0°C, 2.5 h; ii) 2-methoxypropene, PTSA, THF, 30 min; iii) NaOH, MeOH, reflux, 1 hour; iv) NaH, BnBr, DMF, 0°C, 90 min; v)1 M HCl–MeOH, 1:10, rt, 30 min (67%, over 5 steps), vi) NaH, PMBCl, DMF, rt, 12 h (77%); vii) $PdCl_2$, MeOH, rt, 4 h (71%). All = allyl, Piv = $(CH_3)_3CCO$, (Pivaloyl), PMB = *p*–methoxybenzyl, Bn = benzyl.

The diol **10** was easily converted in high yield into **11**, a selectively protected intermediate without the labile *trans* isopropylidene group. In the synthesis of Ins(1,4,5)P_3 analogues *p*-methoxybenzyl groups are often used to mask the hydroxyls designated for phosphorylation towards the end of a synthetic route, because they are easily removed in the presence of other protecting groups such as benzyl ethers[22]. This approach has been adopted here also. Thus, *p*-methoxybenzylation with sodium hydride and *p*-methoxybenzyl chloride in DMF gave the fully protected product **11**. The allyl glycoside was then cleaved by stirring **11** vigorously with palladium chloride in MeOH for 4 h to give **12**. The reaction becomes increasingly acidic as the reaction proceeds, and must be neutralised before work-up to prevent the formation of side products if acid-labile groups such as *p*-methoxybenzyl ethers are present. This method represents a more convenient and higher yielding cleavage of the allyl glycoside than that reported in the literature[76].

3.2.2 Synthesis of the *C*-glycosides

With **12** in hand, attention now turned to the synthesis of the *exo*-methylene derivative. Oxidation of **12** using oxalyl chloride and DMSO in dichloromethane (Swern oxidation) proceeded smoothly to give **13** with a yield of 67%. The IR spectrum displayed the characteristic C=O stretching at 1755 cm^{-1}, while ^{1}H NMR spectrum showed the disappearance of the anomeric proton.

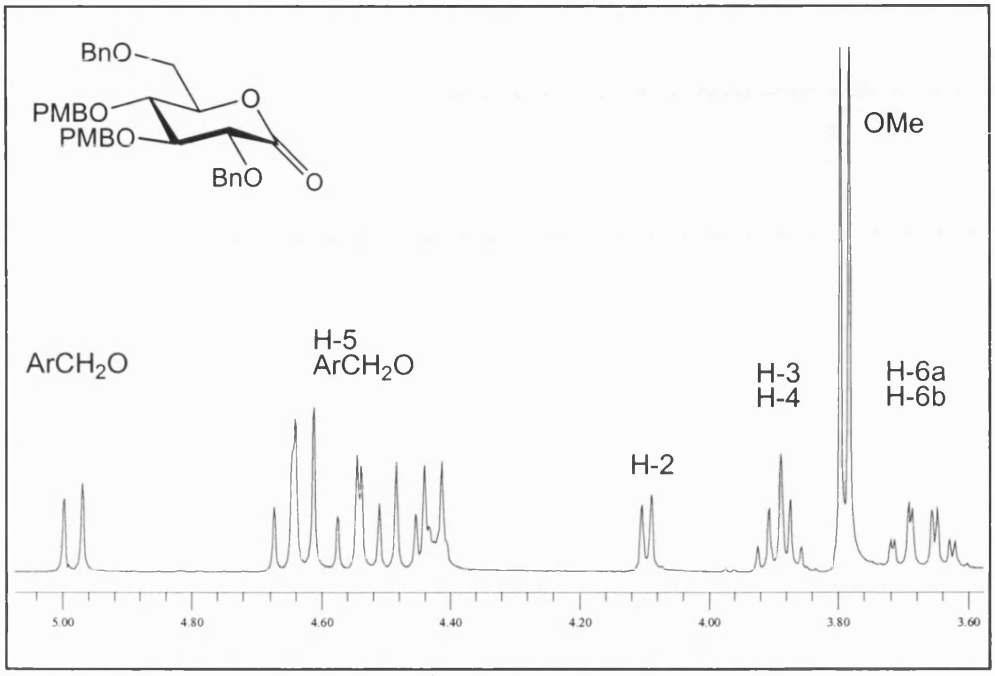
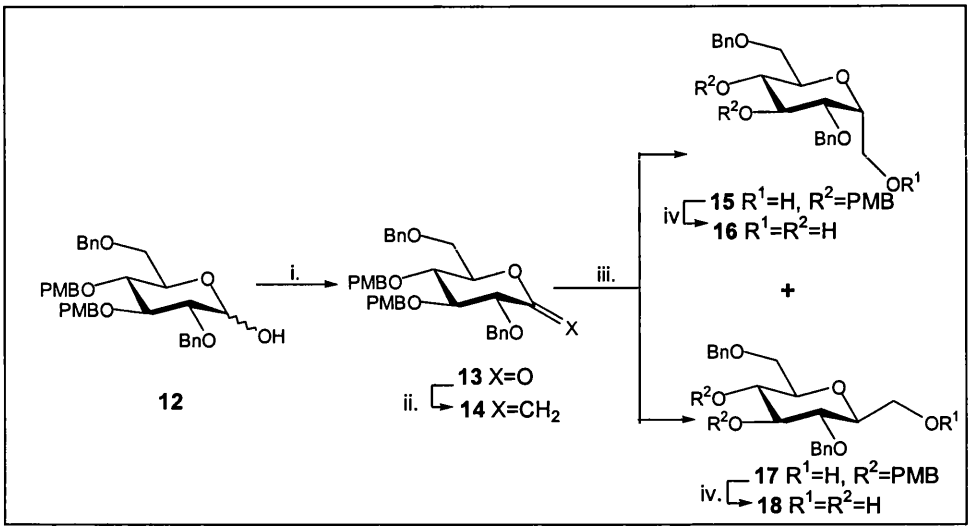


Figure 3.9: Part of the 400 MHz ^{1}H NMR of lactone **13**.

Elaboration to the *exo*-methylene derivative **14** was carried out by the Tebbe reaction[77]. The product was obtained in 65% yield after purification by flash chromatography. The Tebbe reagent was chosen over the Wittig to synthesis the *exo*-methylene sugars as it gives better product yields, particularly when the lactone substrate is hindered[78].

The α and β epimers of the *C*-glucosylmethanol derivatives were then synthesised by hydroboration reactions[77]. Hydroboration using 9-BBN produced β-2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-D-glucopyranosylmethanol (**17**) exclusively. The structure of **17** was difficult to assign from the ^{1}H NMR spectrum due to the protons of the benzyl having a similar influence on the chemical shifts of the protons H-1' and H-6. However a ^{1}H-^{13}C NMR correlation experiment allowed assignment of H-1' and H-6 due to the downfield shift of the benzylated C-6 in the ^{13}C NMR spectrum. A small sample was also converted into the acetate and the resulting downfield shift of the H-1' protons confirmed the assignment. Hydroboration of **14**

with borane-THF complex produced a mixture of α- and β-isomers **15** and **17** in an approximate ratio of 1:2. The two products could not be separated by flash chromatography at this stage.



Scheme 3.2: Route to triols **16** and **18**.
Reagents and conditions: i) a. Oxalyl chloride, CH_2Cl_2, –78 °C, b. Me_2SO added dropwise, c. (**12**) in CH_2Cl_2 added dropwise, d. NEt_3 (67%); ii) Pyridine, THF, Tebbe reagent (1.1eq) added dropwise –45°C for 30 min 65%); iii) 9–BBN, THF 0°C, 3 h (58%) or BH_3-THF, 0°C, 2 h (74%); iv) CF_3COOH, CH_2Cl_2 (80%);

Removal of the PMB protecting groups using 10% TFA in DCM furnished a separable mixture of the corresponding triols **16** and **18** in good yields.

3.2.3 Phosphorylation and Deprotection

The phosphitylating reagent chosen for the synthesis was bis(benzyloxy)diisopropylaminophosphine. This reagent was prepared by the literature procedure[79]. In brief, *N,N*-diisoproylamine was added dropwise to an ethereal solution of phosphorus trichloride at –78°C. The product was isolated and purified by distillation under reduced pressure to give **19** which was stored as a crystalline solid at –20°C. Benzyl alcohol was then added to a solution of **19** in triethylamine and dichloromethane. After purification the phosphitylating reagent was obtained as a clean sample δp 149.03 ppm.

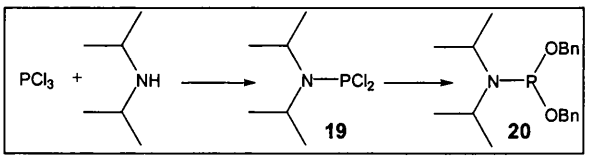
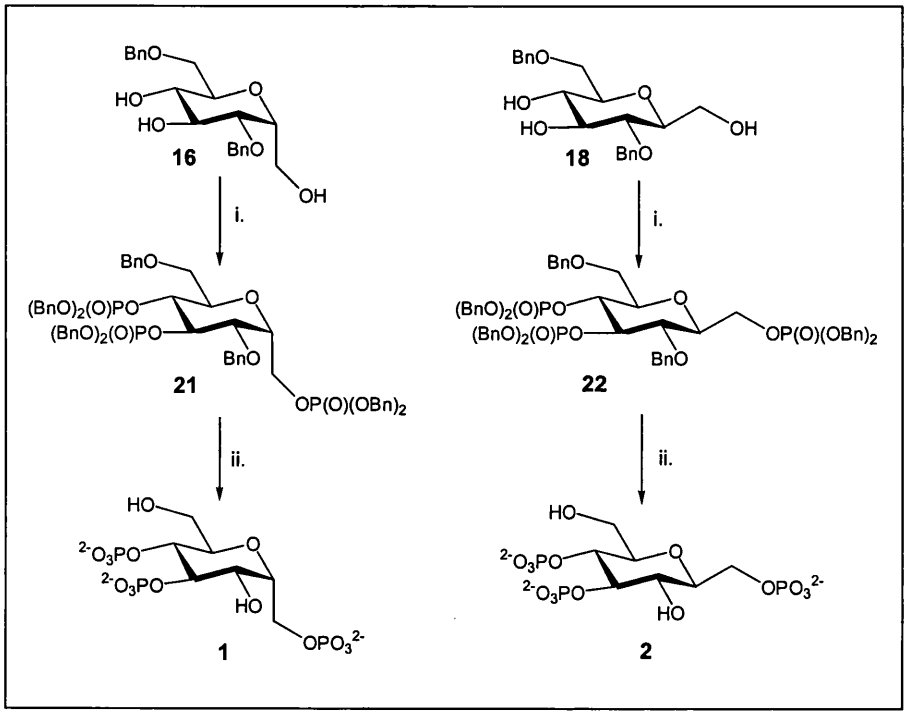


Figure 3.10: Route to the phosphitylating reagent.

The two triols were treated identically in the rest of the synthesis but for the purpose of this discussion when NMR values are quoted it is the α-anomer being referred to. Phosphitylation was carried out at rt using 2 equivalents of phosphitylating reagent per hydroxyl group and three equivalents of 1*H*-tetrazole, in a small volume of dry dichloromethane. A mixture of phosphitylating reagent and 1*H*-tetrazole was stirred for 30 min; the mixture turned cloudy indicating the phosphitylating agent-tetrazolide intermediate had been formed. The triol was added and after 30 min, TLC indicated complete conversion into the trisphosphite. After oxidation with *m*CPBA and work-up, the ^{31}P NMR spectrum of **21** revealed three phosphate signals at 0.21, –0.594 and –1.07ppm, corresponding to the phosphorus atoms of the trisphosphate.

Deblocking of the eight benzyl protecting groups was achieved using hydrogenation over a palladium catalyst. The products were purified using ion-exchange chromatography on Q Sepharose Fast Flow resin eluting with a 0–1 mol dm^{-3} gradient of triethylammonium hydrogen carbonate, pH 7. The phosphate-containing fractions were detected and combined, and the total amount of compound was quantified using a modification of the Briggs phosphate assay[80].



Scheme 3.3: Synthetic route to α and β-*C*-(hydroxymethyl)-1-deoxy-D-glucopyranoside 3,4, 1'-trisphosphate analogues.
Reagents and conditions: i) a. $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, b. *m*CPBA, –78 °C to rt (78%); ii) H_2, Pd–C, 50 psi, MeOH–H_2O, 24 h.

The structure of the product was identified as the required triethylammonium salt of the trisphosphate (**1**) on the basis of its ^{31}P NMR spectrum which showed three signals, and its ^{1}H NMR spectrum in CD_3OD. The accurate negative FAB mass spectrum showed a mass consistent with that predicted for $[M-H]^-$.

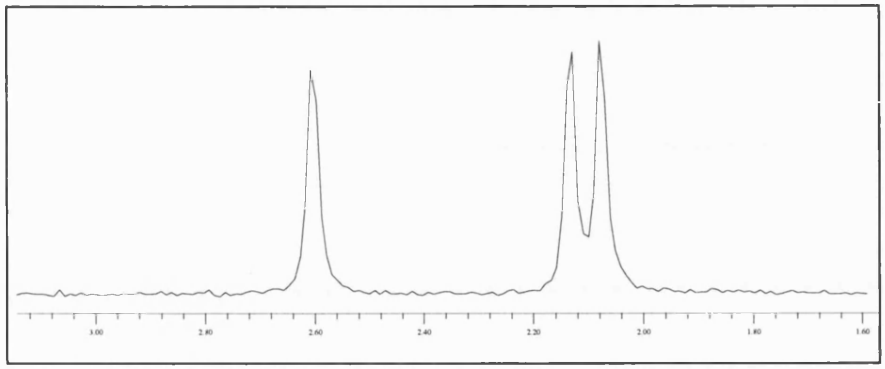


Figure 3.11 : 162 MHz ^{31}P NMR spectrum of the triethylammonium salt of **1** in CD_3OD.

3.3 Biological Results

The ability of a sample of each of the analogues to release Ca^{2+} from permeabilised hepatocytes was compared with that of a sample of Ins(1,4,5)P_3. The results are shown in Table **3.1.**

Maximally effective concentrations (10 μM) of Ins(1,4,5)P_3 and **1** released the same fraction of the intracellular Ca^{2+} stores, 47 ± 2% (n = 5) and 42 ± 5% (n = 3) respectively; half-maximal effects (EC_{50}) occurred with 144 ± 6nM and 2.41 ± 0.17μM, respectively (figure 3.12) . At a concentration of 10 μM, **2** released only 19 ± 3% (n = 5) of the stores. Higher concentrations of **2** were not examined, but its inability to antagonise the response to a submaximal concentration of Ins(1,4,5)P_3 (table **3.2**) suggest that it is not a partial agonist. The results are therefore consistent with **9** being a full agonist with an EC_{50} of about 10μM. Thus, trisphosphate **1** was similar in potency to Gluc(2'3,4)P_3 and the xylose equivalent, while **2** was considerably weaker, this will be discussed below.

	EC_{50}	*h*	% release with 10μM	n
$Ins(1,4,5)P_3$	144 ± 6 nM	1.68 ± 0.26	47 ± 2	5
1	2414 ± 173 nM	4.99 ± 1.84	42 ± 5	3
2	nd	nd	19 ± 3	5
$Gluc(2'3,4)P_3$ [a]	1867 ± 64 nM	2.76 ± 0.10	47 ± 3	3

Table 3.1: $^{45}Ca^{2+}$ release data for $Ins(1,4,5)P_3$, $Gluc(2'3,4)P_3$, **1** and **2** from permeabilised hepatocytes.
The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to logistic equations. Results are shown as means ± S.E.M.
[a] Data for $Gluc(2'3,4)P_3$ obtained in an independent study [54] when $Ins(1,4,5)P_3$ had EC_{50} value and Hill coefficients (*h*) of 153 ± 11 nM and 2.25 ± 0.20 nM respectively.

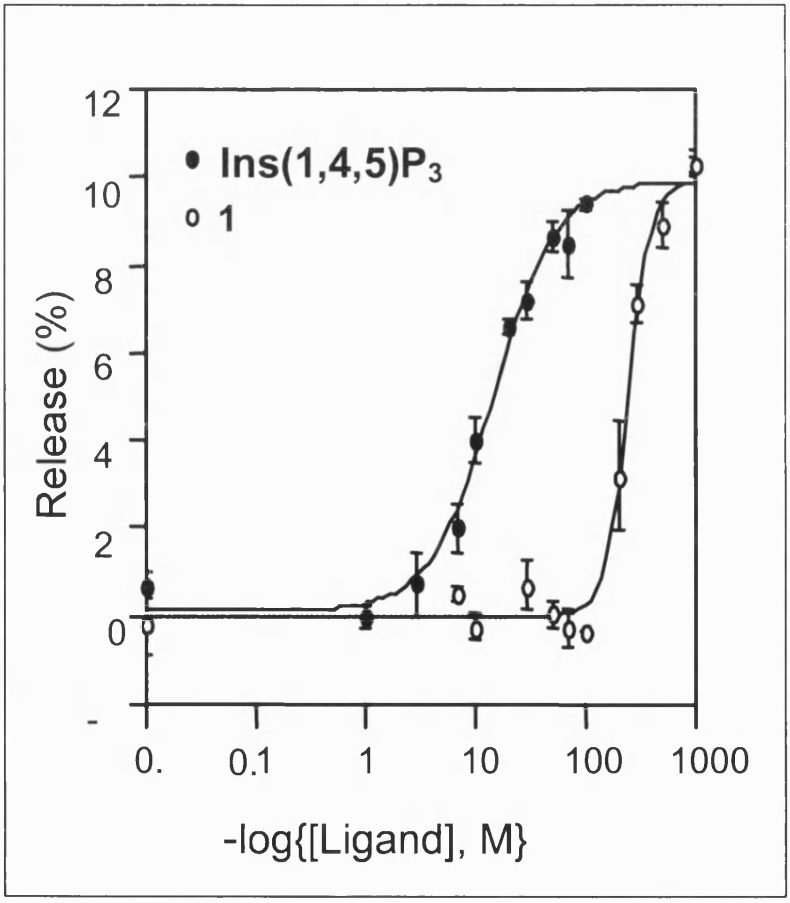


Figure 3.12: Ca^{2+} mobilization evoked by $Ins(1,4,5)P_3$ and **1** from permeabilised hepatocytes.

	% Ca^{2+} release
$Ins(1,4,5)P_3$ (150 nM)	22 ± 4
2 (10 μM)	21 ± 2
with **2** (10 μM)	38 ± 1

Table 3.2: $^{45}Ca^{2+}$ release data for combined stimulation with $Ins(1,4,5)P_3$ and **2** from permeabilised hepatocytes.
The percentage of the intracellular Ca^{2+} stores released by a submaximal concentration of $Ins(1,4,5)P_3$ alone or in combination with **2** are shown. Results are shown as means ± S.E.M for 3 independent experiments.

3.4 Conclusions

Previous studies [54;56] have clearly demonstrated that sugar-based polyphosphates (see chapter 4) are able to approach the potency of $Ins(1,4,5)P_3$ but not of adenophostin. Such molecules possess a glucopyranosyl 3,4-bisphosphate with an auxiliary phosphate which is accommodated in an optimal position for binding using a second ring as in adenophostin. A challenge therefore is to explore the possibility of optimal positioning of the third phosphate without the need for the second ring. This would also be useful in the design of related molecules since it would avoid problems of coupling strategies (see chapters 4 and 5).

It is encouraging to see that the *C*-glycoside mimics prepared here (**1** and **2**) do possess $Ins(1,4,5)P_3$ like Ca^{2+} mobilising activity, although neither has attained the activity of disaccharide analogues (chapter 4). The differential potencies of **1** and **2** are of interest and indicate that **1** presents the auxiliary phosphate group in a more favourable position. However, a comparison of the activity of **1** with that of $Glc(2'3,4)P_3$ (figure 3.2) indicates that both molecules possess similar potency, implying no significant advantage of **1** over $Glc(2'3,4)P_3$ in binding to the $Ins(1,4,5)P_3$ receptor.

Moitessier *et al* [49] synthesised 2′, 3, 4-trisphosphates of (2-hydroxyethyl) α and β-D-xylopyranosides and 3′, 3, 4-trisphosphates of (2-hydroxypropyl) α and β-D-xylopyranosides. It was found that three of the mimics were comparable and released approximately the same amount of intracellular Ca^{2+}, roughly with ten fold lower potency than $Ins(1,4,5)P_3$, with only the larger and more flexible β-hydroxypropyl mimic having a much lower potency. On the basis of these results it

might be reasoned that the *C*-glycosides with a less flexible chain should both behave similarly while, based upon the structure of Ins(1,4,5)P_3 one might expect the β modified *C*-glycoside to have higher potency. However, on examination of molecular models this is clearly not the case. The differential activity of **1** and **2** can be qualitatively explained by the fact that the α-*C*-glycoside phosphate group in **1** could readily access some of the conformational space described by the 1-phosphate of Ins(1,4,5)P_3. While this is not impossible for the β-*C*-glycoside phosphate group in **2**, it does seem to be more difficult and we also cannot exclude a potentially disfavour able steric or electronic interaction of the extra CH_2 group with the receptor protein. The results show that the anomeric oxygen atom in Glc(2'3,4)P_3 is not essential for biological activity and confirm that the three-dimensional location of the third phosphate group plays an important role in strong binding to the receptor.

These *C*-glycoside based polyphosphate analogues represent steps in designing high potency ligands using insights gained from the adenophostins and are worthy of further development.

3.5 Further C-glycoside analogues.

Several other *C*-glycoside analogues have been reported by the Shuto group since this work was carried out. Compounds **3.4** and **3.5** have only been reported as a communication[81]. Although the biological evaluation of these compounds has not been reported, the subsequent paper[82] where the synthesis of **3.6** was described suggests that the length of the side-chain in **3.4** and **3.5** may be too long to allow the third phosphate group to achieve an efficient position for binding to the Ins(1,4,5)P_3R.

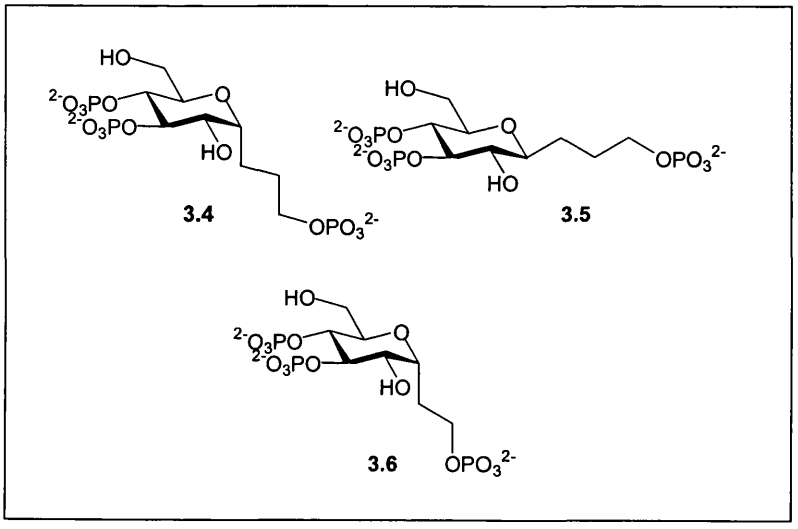


Figure 3.13: Further *C*-glycosides.

The binding affinity of **3.6** to the Ins(1,4,5)P_3R in calf cerebellum was found to be only about 2-fold lower than Ins(1,4,5)P_3. It is worth noting that this is considerably better than the *O*-glycoside equivalent [Gluc(2'3,4)P_3]. The different activity between the *C*-glycoside and Gluc(2'3,4)P_3 may not be due to the property of the glycosidic linkage, since the side-chain length of **3.6** is also different from Gluc(2'3,4)P_3.

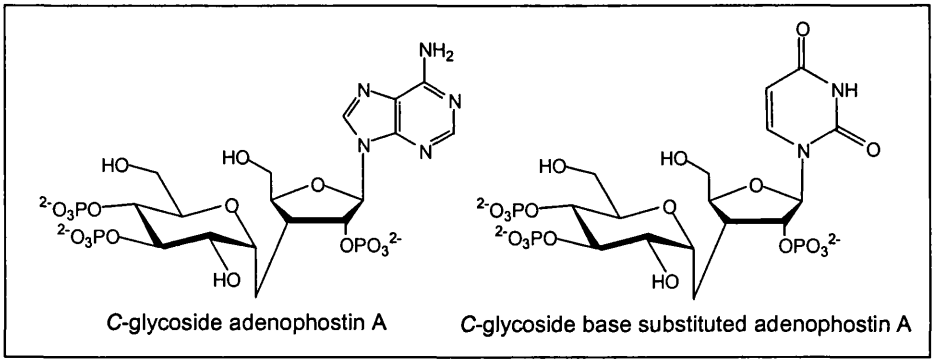


Figure 3.14: *C*-glycoside adenophostin A derivatives.

Finally, the same group has prepared a *C*-glycosidic analogue of adenophostin A and an *C*-glycoside analogue where the purine motif has been replaced by a much smaller pyrimidine (uracil) (Figure 3.14)[83]. The ability of the compounds to release Ca^{2+} from permeabilised hepatocytes was compared. The *C*-glycosidic adenophostin A was the most active compound with EC_{50} of 77 ± 7 nM while the base substituted adenophostin A was dramatically lower (378 ± 87 nM)[84]. As the *C*-glycosidic adenophostin A is less active than adenophostin A it suggests that the oxygen may influence the relative position of the 2'-phosphate, due to the steric and electronic influence near the 1"-position.

Chapter Four

Disaccharide analogues

Chapter 4

4 Disaccharide analogues

4.1 Introduction

As already discussed in chapter 3, minimal structure analogues were designed and synthesised to determine whether the adenine component of the adenophostins was essential for activity. All the monosaccharide analogues had lower affinity than $Ins(1,4,5)P_3$; This lower affinity was thought to be due, at least in part, to the conformational flexibility of the ethylphosphate side chain in figure 4.1 [48]. Consequently, various phosphorylated disaccharides incorporating a D-glucopyranosyl 3,4-bisphosphate moiety with an α-glycosidic linkage to a second sugar containing one or more phosphates were designed and synthesised.

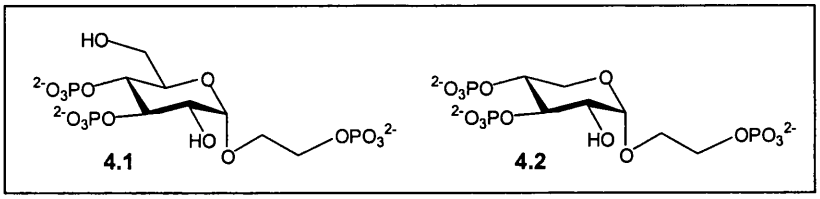


Figure 4.1: $Glc(2',3,4)P_3$ (**4.1**) and the xylopyranoside equivalent (**4.2**).

The first series of disaccharide polyphosphates [54] were based on α,α'-trehalose and sucrose, both of which are readily available naturally occurring starting materials (figure 4.2). All three of the synthetic analogues, sucrose 3,4,3'-trisphosphate [$Sucr(3,4,3')P_3$], α,α'-trehalose 3,4,3',4'-tetrakisphosphate [$Trehal(3,4,3',4')P_4$] and α,α'-trehalose 2,4,3',4'-tetrakisphosphate [$Trehal(2,4,3',4')\ P_4$], contain the same phosphorylated glucose component, identical to that found in the adenophostins. The third phosphate was on a second ring in a more rigid conformation than in $Glc(2',3,4)P_3$. Although molecular modelling indicated that the positioning of the third phosphate may not have been ideal, these were easily accessible from existing disaccharides avoiding the need for difficult glycosidation coupling reactions.

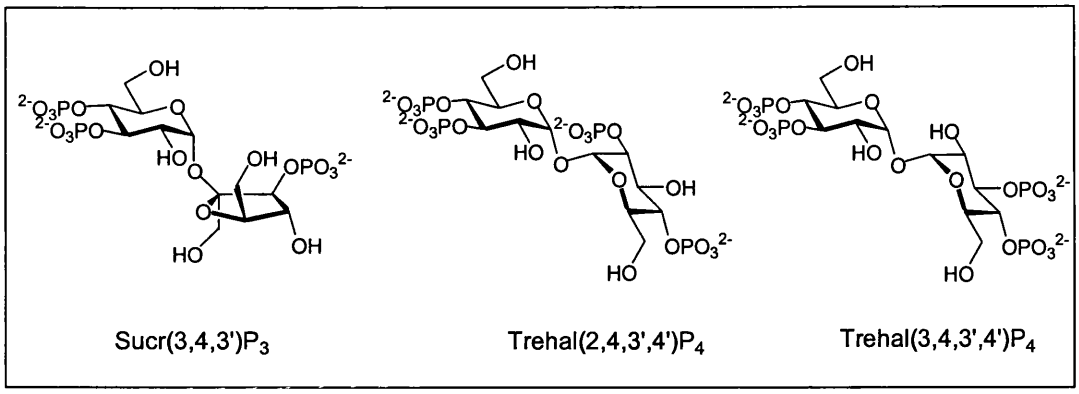


Figure 4.2: Disaaccharide polyphosphates.

These analogues together with adenophostin A, Ins(1,4,5)P_3 and Glc(2',3,4)P_3 underwent biological testing to determine their potencies in both [^{3}H]Ins(1,4,5)P_3 binding and $^{45}Ca^{2+}$ mobilisation from rat hepatocytes. They had the following rank order which was the same in both binding assays and $^{45}Ca^{2+}$ release, adenophostin A>Ins(1,4,5)P_3>Trehal(2,4,3',4')P_4> Glc(2',3,4)P_3≈Trehal(3,4,3',4')P_4>Sucr(3,4,3')P_3.

Trehal(2,4,3',4')P_4 is an asymmetrical regioisomer of Trehal(3,4,3',4')P_4 in which a single phosphate is relocated to a position two carbons removed from the glycosidic oxygen. Trehal(2,4,3',4')P_4, gave the better result with a potency 10-fold lower than Ins(1,4,5)P_3, suggesting that the positioning of the 2-phosphate group must be better than either of the 3 or 4-phosphates. It was also reasoned that the alteration in the substitution pattern on one glucose residue may influence the conformation about the glycosidic linkage.

These findings together with the biological data from Glc(2',3,4)P_3 suggested that the precise orientation and position of the phosphate group needed to be controlled in some way. On the basis of these considerations, methyl 3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside 2,3',4' trisphosphate (ribophostin figure 4.3) [54;55] was designed to restrict the conformation of the side chain of Glc(2',3,4)P_3 by introducing a ribofuranoside ring, as in the adenophostins. The potency of ribophostin in Ca^{2+} release assays was similar to that of Ins(1,4,5)P_3, but still about 20-fold less than that of adenophostin A. The lower affinity of ribophostin suggests that the adenine component of the adenophostins is not essential for activity, but enhances affinity for the Ins(1,4,5)P_3 receptor.

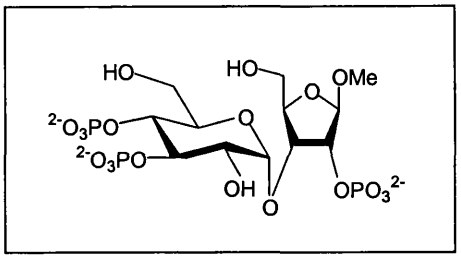


Figure 4.3: Ribophostin.

Sucr(3,4,3')P_3 was found to be 25-fold less potent in Ca^{2+} release than ribophostin, which is surprising as it is structurally quite similar to ribophostin. The authors suggested several reasons why it should be so much weaker; i). Steric hindrance from one or both hydroxymethyl groups on the fructofuranoside in Sucr(3,4,3')P_3 interfering with binding. ii) Overlapping anomeric effects about the glycosidic linkage, not present in ribophostin, influence the conformation about the glycosidic linkage. iii). The presence of a quaternary furanosyl anomeric centre may lead to increased flexibility about the fructofuranosyl linkage.

Simplification of ribophostin led to the synthesis of 1-*O*-[(3'*S*,4'*R*)-3-hydroxytetrahydrofuran-4-yl]-*α*-*D*-glucopyranoside 3,4,3'-trisphosphate (furanophostin, figure 4.4)[57] which lacks both the *O*-methyl and the 4-hydroxymethyl moieties but retains the rigidity of the five-membered ring. Again, the activity of furanophostin was found to be similar to Ins(1,4,5)P_3 and to ribophostin in Ca^{2+} release assays.

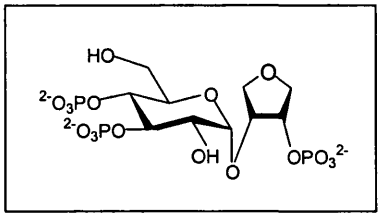


Figure 4.4: Furanophostin.

At a similar time the Matsuda group published the synthesis of furanophostin[56] and some related compounds shown in figure 4.5. Biological testing of furanophostin in binding to porcine cerebellum, showed a comparable affinity to Ins(1,4,5)P_3 itself in this assay, thus confirming the conclusions from the $^{45}Ca^{2+}$ release assays, that furanophostin is almost equipotent to Ins(1,4,5)P_3. **4.3** Has a structure midway between furanophostin and ribophostin; it contains a hydroxymethyl group analogous to the ribophostin 4-hydroxymethyl, but like furanophostin lacks the 1-*O*-methyl group. It has a similar binding affinity to furanophostin indicating that the 4-hydroxymethyl motif does not increase affinity for the Ins(1,4,5)P_3 receptor towards that of adenophostin A, and neither

does it seem necessary for Ins(1,4,5)P_3-like activity. Compound **4.4** which lacks the third phosphate group of **4.3**, was, as expected on parallel studies of Ins(1,4,5)P_3 almost inactive. Compound **4.5,** a regioisomer of **4.3**, showed about 20-fold lower potency than **4.3**, which suggested that the binding of the compounds depends on the three-dimensional location of the third phosphate group.

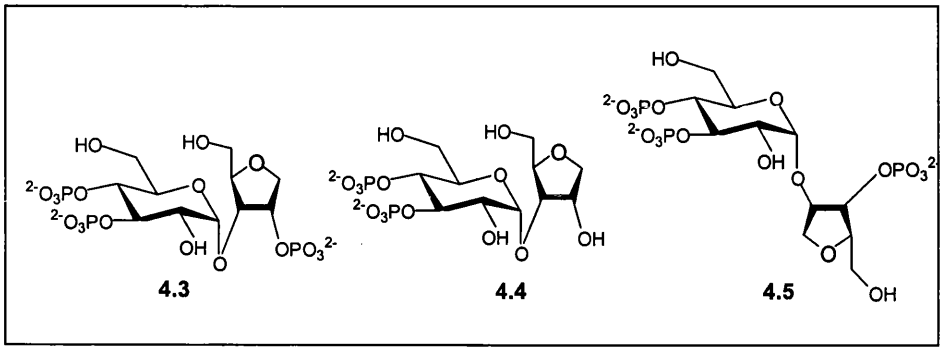


Figure 4.5: Furanophostin-related disaccharide polyphosphates.

In the last chapter we designed and synthesised α and β-D-glucopyranosylmethanol 3,4,1'-trisphosphate analogues based on Gluc(2',3,4)P_3 but with a shorter side chain (chapter 3)[85]. In these molecules the third phosphate group is attached to a carbon centre fixed in the α and β-positions. However, biological evaluation showed that the α-*C*-analogue was only comparable to Gluc(2',3,4)P_3, while the β-*C*-analogue was even weaker. This finding suggests that the second ring of ribophostin and furanophostin is required for greater affinity.

4.2 Synthesis of xylofuranophostin and its diastereoisomers.

In the present work we have taken the simplification of furanophostin a step further by replacing the glucopyranosyl 3,4-bisphosphate structure in furanophostin with the xylopyranosyl equivalent to give **23.** In order to explore the effect of stereochemical variations on the biological activity of **23**, and in particular the positioning of the non-vicinal phosphate group for potent activity, we have also synthesised three of its diastereoisomers **24**, **25** and **26**. The series of trisphosphates **23** to **26** may be regarded as conformationally restricted analogues of the original xylopyranoside-based[49] analogue **4.2** (figure 4.1) and its β-linked equivalent.

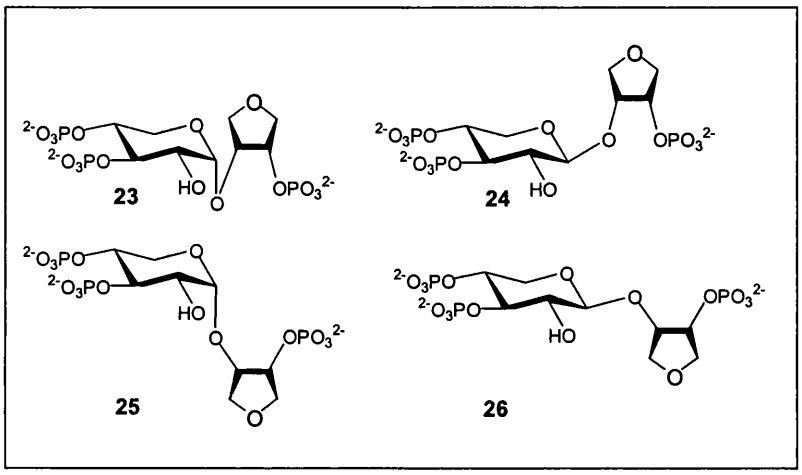


Figure 4.6: Xylofuranophostin and its diastereoisomers.

4.2.1 Discussion

We planned to synthesise the target compounds using glycosidation reactions with glycosyl donor and tetrahydrofuran derivatives shown in scheme 4.2. A typical route is shown in figure 4.7. We designed a phosphite donor as it is easy to prepare in quantitative yield and a colleague (Dr. R. D. Marwood) has successfully employed the analogous glucose phosphite donor in the synthesis of furanophostin.

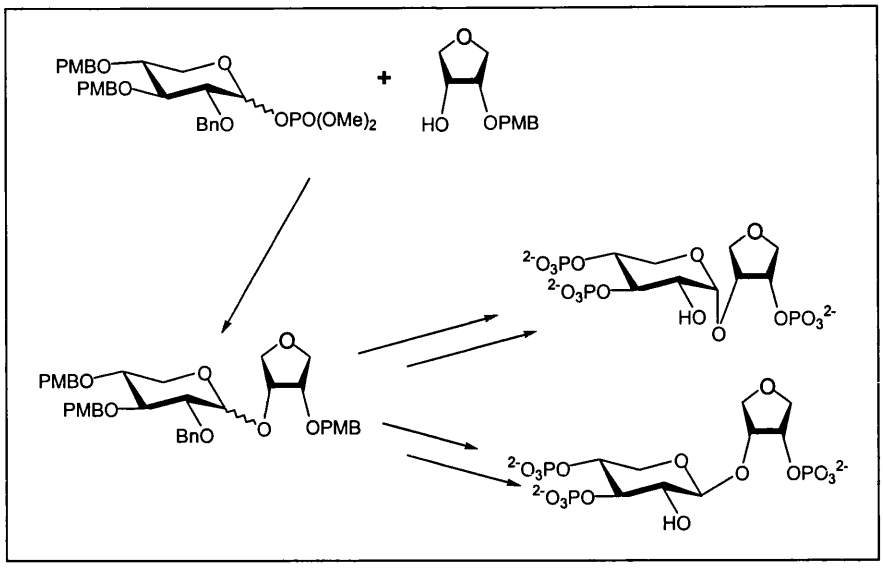


Figure 4.7: Route to xylofuranophostin and its β-epimer.

4.2.2 Synthesis of the donor

Although the required regioselectively-protected D-xylopyranose **33** had already been prepared and reported[76], the overall yield from allyl α-D-xylopyranoside (**27**) was restricted by the unavoidable formation of two butane diacetal protected regioisomers in the first step of the synthetic route.

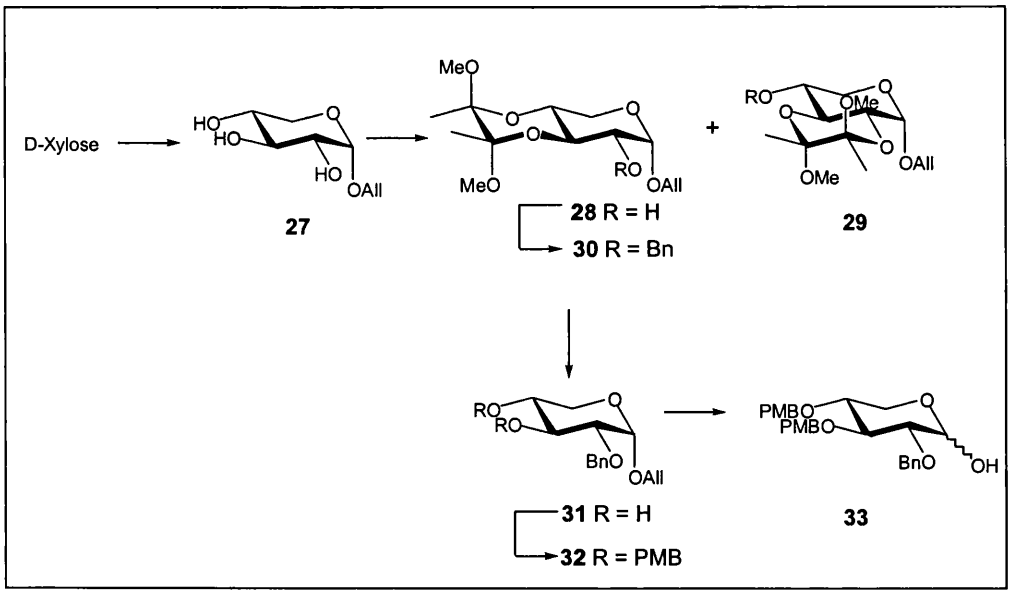


Figure 4.8: Literature route to fully protected D-xylopyranose.

A different route was therefore investigated, with the aim of increasing the overall yield of **27** while reducing the need for time-consuming purifications by column chromatography. Thus, Fischer glycosidation of xylose by a modification of the previously described method [76] gave the allyl xyloside (**27**) as colourless crystals in 41% yield over three crops. It was found that the reaction mixture could be satisfactorily neutralised by addition of solid $NaHCO_3$, avoiding the need for more expensive Amberlite IR-45 (OH^-) resin. Furthermore, adding diisopropyl ether to the crystallisation mixture immediately before filtration facilitated the isolation of the crystalline product and consequently the yield was significantly improved from that reported of 28% to 41%.

The next step involved the selective acylation at position 2 of xylose using the trimethylacetyl chloride (pivaloyl chloride). Selective protection of alcohols to give the corresponding pivaloyl esters has mainly been used for esterifying the primary hydroxyl groups in the presence of secondary hydroxyl groups. A literature search revealed that the 2-OH is the most reactive of all the secondary hydroxyl functions in the *α-D*-glucopyranosides. It was therefore reasoned that the 2-OH in the xylose derivative would also be the most reactive[73]. Therefore, a modification of a selective pivaloylation method described in this report was used to selectively acylate the 2-hydroxyl of **27**. After several systematic changes relating to equivalents of pivaloyl chloride, temperature and reaction time, the best conditions were established. Thus, treatment of **27** with pivaloyl chloride at –20°C in pyridine did indeed give one major product, although other minor products were shown to be present by TLC, presumably the di-pivaloyl esters. The required 2-*O*-pivaloyl ester **34** could be isolated by crystallisation in 53% yield, without the need for purification by chromatography. The major product was identified as 2-*O*-pivaloyl derivative from the ^1H-^1H COSY NMR spectrum, which indicated a typically deshielded doublet of doublets at δ_H 4.62 with coupling constants J 3.7 Hz and 9.9 Hz, corresponding to H-2.

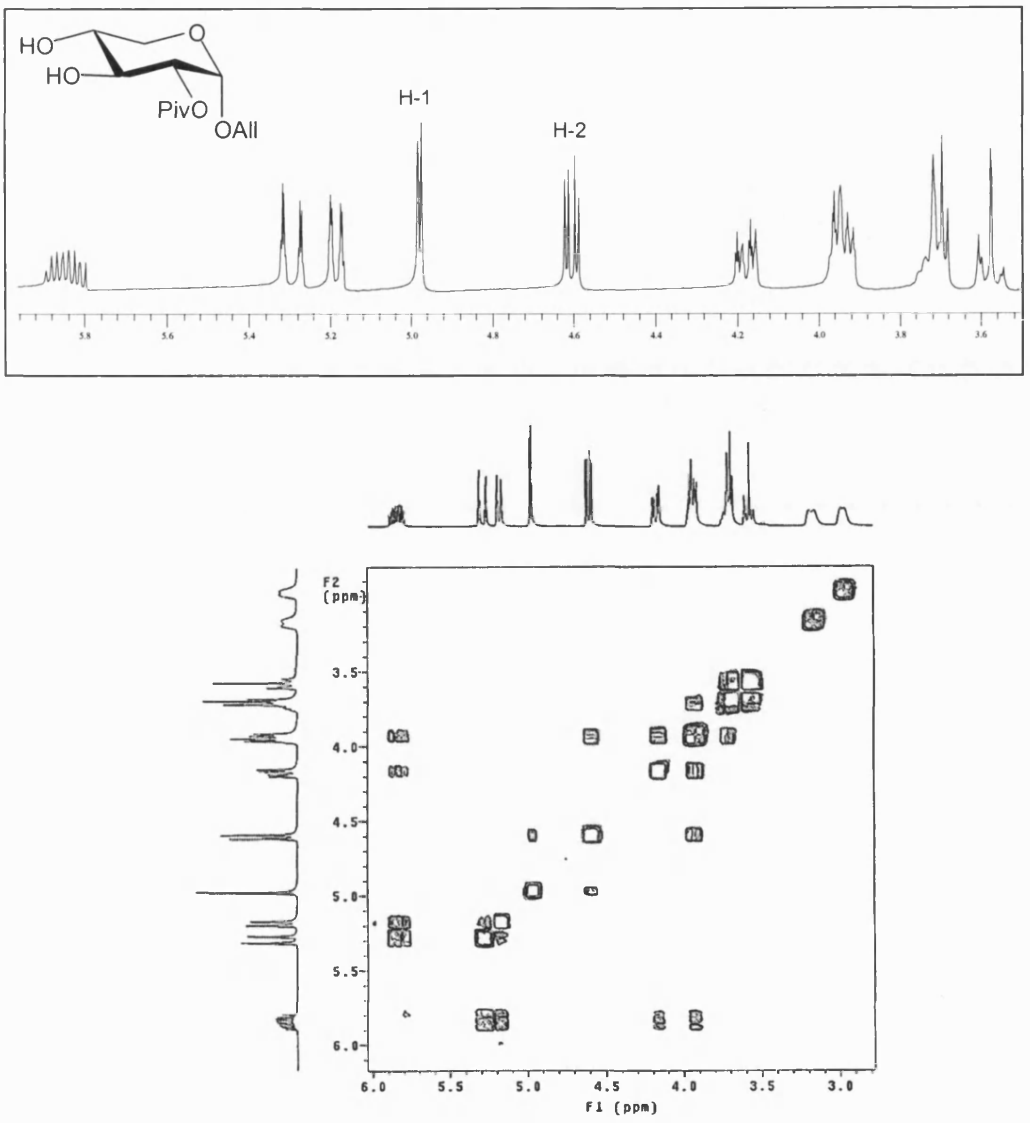


Figure 4.9: Part of 400 MHz ^{1}H and 2D ^{1}H COSY NMR spectra of **34** indicating the deshielded acylated position of H-2.

As in chapter 2, for the synthesis of allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside, the protection of the remaining *trans*-diequatorial hydroxyl groups at positions 3 and 4 was initially achieved by refluxing **34** with butane-2,3-dione, catalytic camphorsulphonic acid and excess trimethyl orthoformate for 3 h. This gave one major product (**35**), which, after purification, was identified as the corresponding butane diacetal (BDA) in 86% yield. Unfortunately, when the reaction was scaled up the yield reduced dramatically due to the migration of the pivaloyl groups under acidic conditions. After the pivaloyl groups were removed by NaOH, the overall yield of the required product was poor, 34% over two steps.

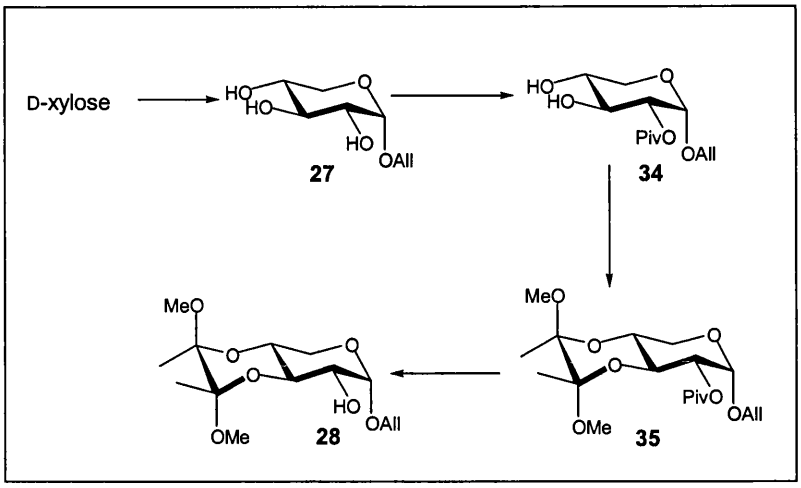
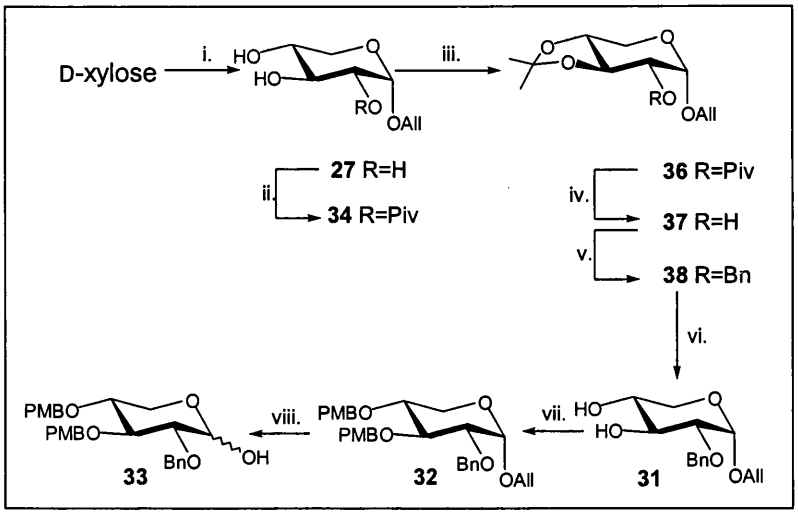


Figure 4.10: Route to BDA derivative **28**.

The same strategy was then adopted as in chapter 3. Thus the *trans*-diequatorial hydroxyl groups at positions 3 and 4 were protected using 2-methoxypropene in THF in the presence of a catalytic amount of *p*-toluenesulphonic acid to give one major product. Since a colleague (Dr. R. D. Marwood) carried out this reaction at a similar time and identified the major product to be the 3,4-*O*-isopropylidene derivative, the crude material obtained from the reaction was carried forward to the next step.

Conversion into the known allyl 2-*O*-benzyl-α-D-xylopyranoside was then straightforward, with each intermediate being isolated for identification. The pivaloyl ester was removed using saponification by heating at reflux with NaOH pellets in methanol to give the free 2-hydroxyl. Benzylation of the 2-hydroxyl was achieved using sodium hydride and benzyl bromide in DMF. Finally, the isopropylidene group was removed by stirring **38** with 1 M HCl in MeOH for 30 min to give allyl 2-*O*-benzyl-*α*-*D*-xylopyranoside (**31**) in high yield (76% over the 4 steps from **27**) after purification by flash chromatography followed by crystallisation. **31** Has been prepared previously but was reported to be an oil[76].



Scheme 4.1: Synthetic route to the D-xylopyranose intermediate.
Reagents and conditions: i) AllOH, HCl, reflux, 16 h; ii) $(CH_3)_3COCl$, pyridine, –40 °C, 2.5 h; (53%); iii) 2-methoxypropene, PTSA, THF, 30 min; iv) NaOH, MeOH, reflux, 1 h; v) NaH, BnBr, DMF, 0 °C, 90 min; vi)1 M HCl (10%), MeOH, rt, 30 min (76%, over 4 steps), vii) a) NaH, PMBCl, DMF, rt, 12 h (84%); viii) $PdCl_2$, MeOH, 0 °C to room temp, 4 h (90%). All = allyl, Piv = $(CH_3)_3CCO$, (Pivaloyl), PMB = *p*-methoxybenzyl, Bn = benzyl

The diol **31** was easily converted in high yield into 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-α,β-D-xylopyranose (**32**), a selectively protected intermediate without the labile *trans* isopropylidene group. Thus, reaction with sodium hydride and *p*-methoxybenzyl chloride in DMF gave the fully protected product. The allyl protection at the anomeric position was then removed by stirring vigorously with a catalytic amount of palladium chloride in MeOH to give xylopyranose **33**. Finally, **33** was converted into the phosphite donor (**39**) with 1*H*-tetrazole and bis(methoxy)(diethylamino) phosphine in dichloromethane, which was used without further purifcation.

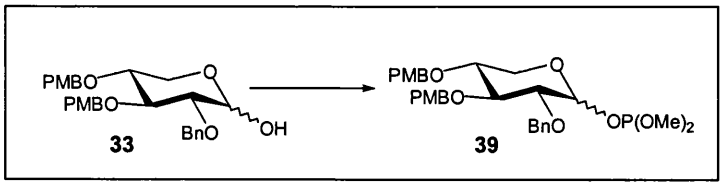
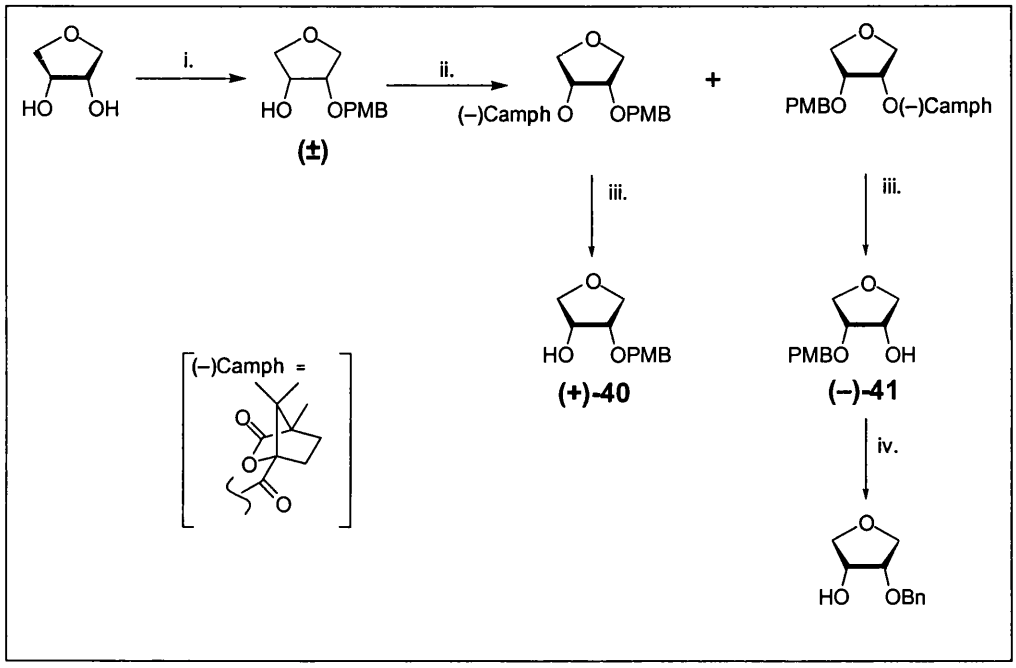


Figure 4.11: Conversion to the phosphite donor.

4.2.3 Synthesis of the acceptor

The acceptors (**40** and **41**) were prepared by Dr. A. M. Riley. Although the structure is simple, the synthesis required optical resolution of an enantiomeric intermediate, and the conversion of one enantiomer into a compound for which the absolute configuration was already known. The route followed is outlined in scheme **4.2**.

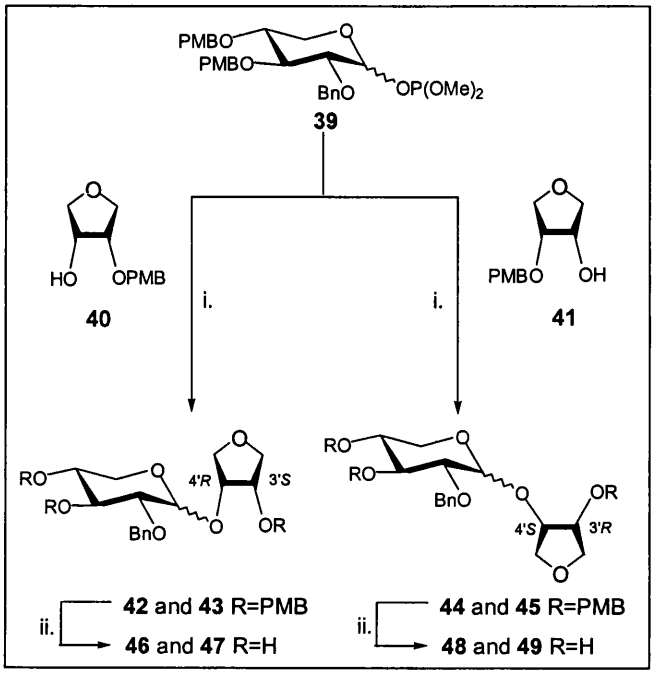


Scheme 4.2: Synthetic route to glycosyl acceptors **(+)-40 and (–)-41**.
Reagents and conditions: i) a) *p*-methoxybenzylidene dimethyl acetal (1.05 equiv.), *p*TSA, DMF, 70 °C; b) DIBAL-H (2.5 equiv.), CH_2Cl_2, –78 °C; 90 % yield for two steps; ii) (–)-(*S*)-camphanic chloride, pyridine, 0 °C to rt, (80 % yield; iii) NaOH, MeOH, reflux, 94–97 %; iv) a) NaH, BnBr, DMF; b) CF_3COOH, CH_2Cl_2; 87 % yield for two steps.

4.2.4 Glycosidation and deprotection

With suitable glycosyl phosphite donor and acceptors in hand, the glycosidation was carried out under established conditions favouring α-stereoselectivity i.e. room temperature in a mixture of 1,4-dioxane and toluene[86]. The crude glycosyl phosphite was coupled to each of the two enantiomeric alcohols in the above manner using zinc chloride and silver perchlorate as promoters. The glycosylation [87] of (+)-(3*R*,4*S*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol (**40**) [20] and of (–)-(3*S*,4*R*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol (**41**) [57] with the di-OMe phosphite gave a

total of four diastereoisomeric products **42–45**. It is interesting to note that, in each case, a mixture of α and β-coupled products was formed using this xylopyranosyl phosphite glycosyl donor, whereas only the α-coupled product was detected when the same conditions were applied to the analogous glucopyranosyl phosphite in the synthesis of furanophostin [57].



Scheme 4.3: Synthetic route to the diastereoisomers.
Reagents and conditions: i) $AgClO_4$, $ZnCl_2$, dioxane, toluene, 4Å sieves, glycosyl acceptor, (70–76%); ii) CF_3COOH, CH_2Cl_2 (18–34%);

The α and β-epimers could not be separated at this stage, therefore it was decided to proceed with the deprotection of the *p*-methoxybenzyl groups in the hope that the anomeric mixture of triols could be separated. Thus, the PMB ethers where removed using 10% trifluoroacetic acid in dichloromethane. In both cases TLC showed the appearance of two new spots, allowing the chromatographic separation of the 4'S,3'R anomers (**48** and **49**). Subsequent crystallisation furnished the pure triols for phosphorylation. Unfortunately, compounds **46** and **47** could still not be fully separated, so the mixture of products was converted into its corresponding acetate by reaction with acetic anhydride and pyridine. The products were then separated by flash chromatography to give the α and β analogues. The β product was further purified by crystallisation. **51** Was then deprotected by stirring with methanolic ammonia overnight and purified by flash chromatography to give the pure triol for phosphorylation.

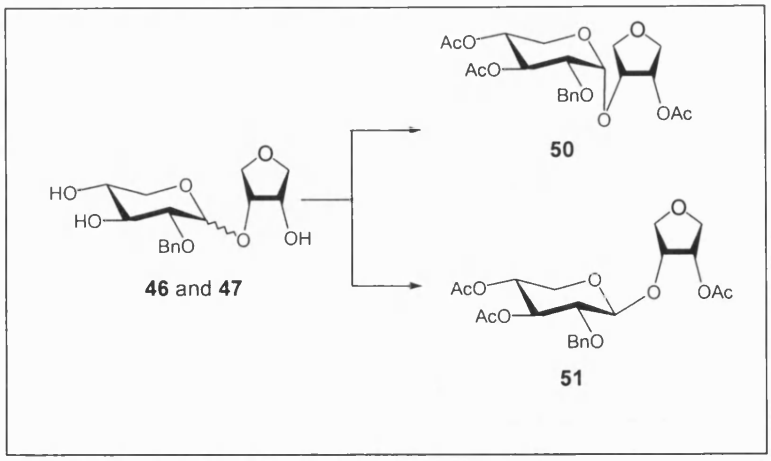


Figure 4.12: Separation of α and β diastereoisomers.

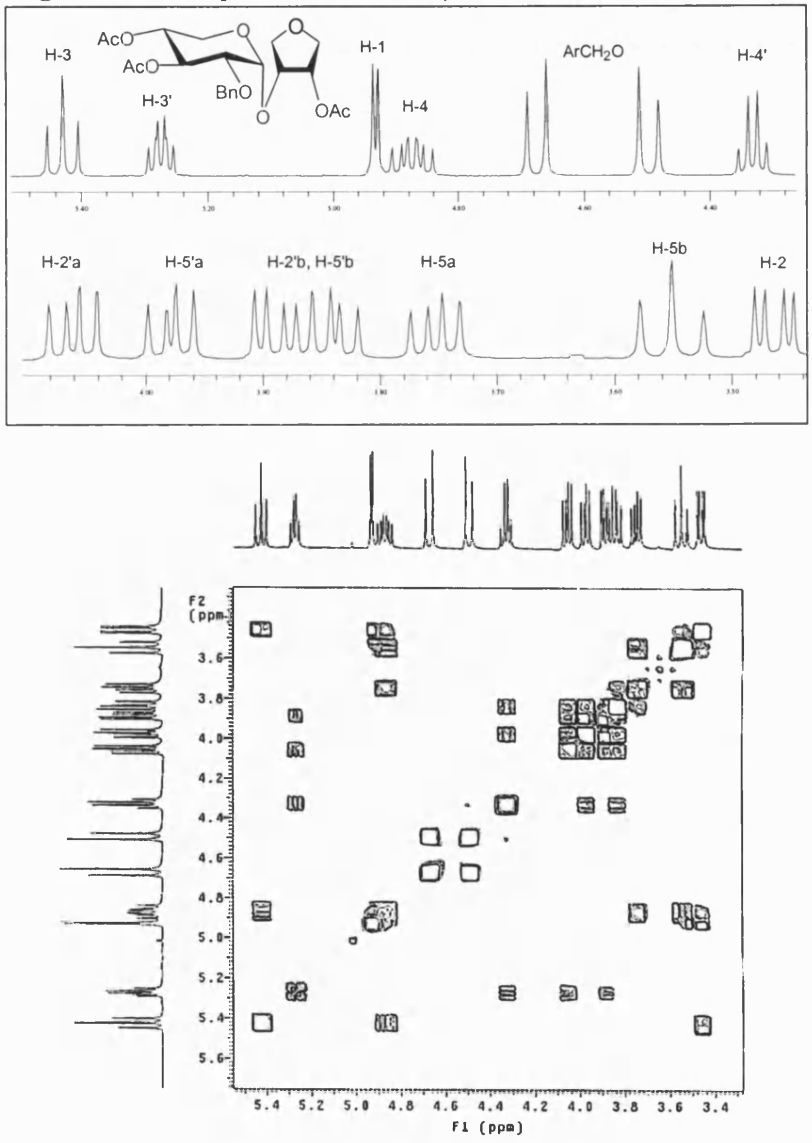


Figure 4.16: Part of 400 MHz ^{1}H and 2D ^{1}H COSY NMR spectra of **50**.

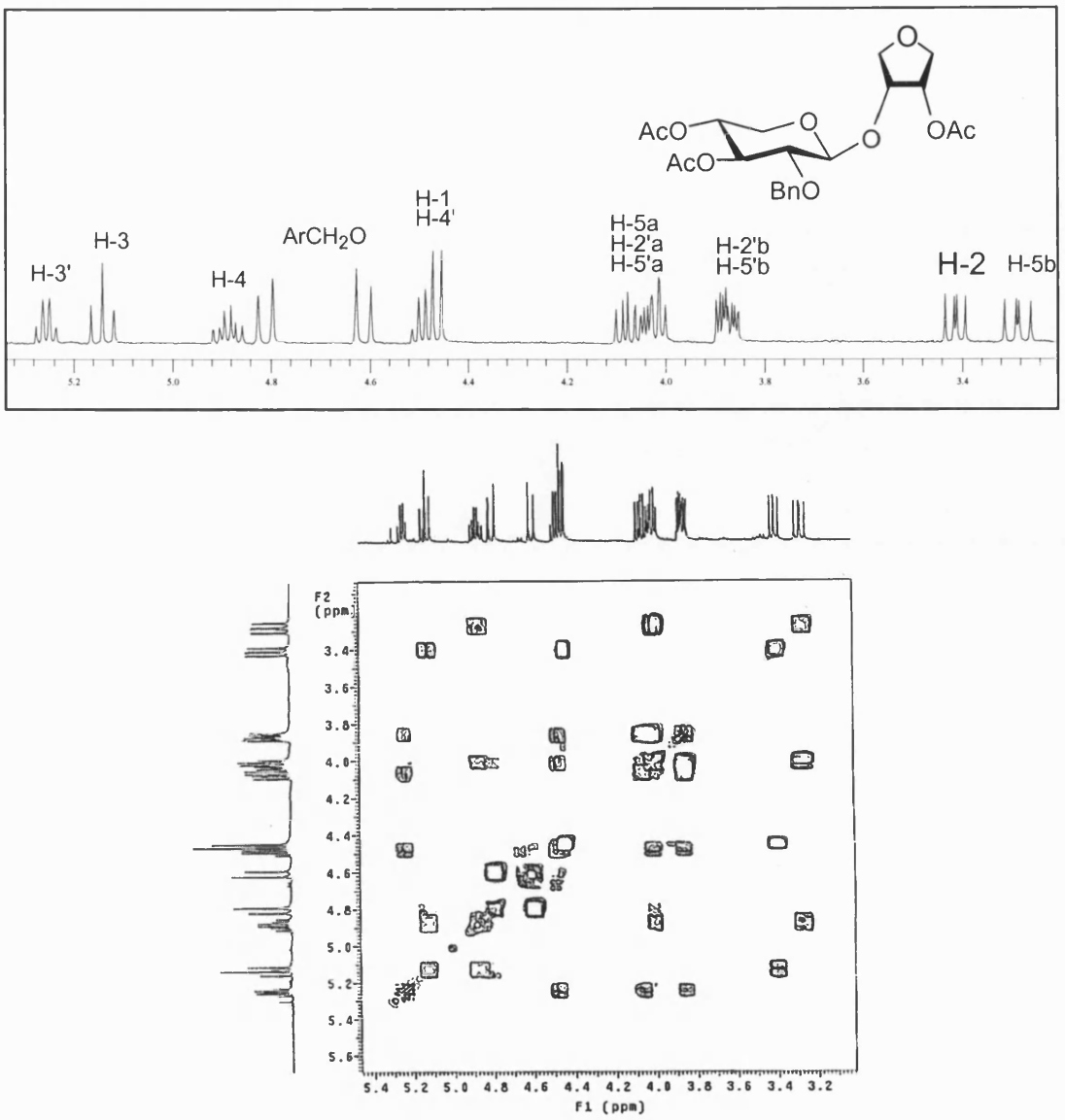


Figure 4.13: Part of 400 MHz ^{1}H and 2D ^{1}H COSY NMR spectra of **51**.

The ^{1}H-^{1}H NMR spectrum of **50** exhibited a doublet at 4.92 ppm with a characteristically axial-equatorial coupling constant of *J* 3.2 Hz, while the ^{1}H-^{1}H NMR spectrum of **51** exhibited a deshielded doublet at 4.44 ppm. There was no sign of cross over contamination by the other isomer in either NMR (Figures 4.12 and 4.13).

4.2.5 Phosphorylation and Deprotection

Phosphitylation of each triol was achieved in the usual way with bis(benzyloxy) (diisopropylamino)phosphine and 1*H*-tetrazole in dichloromethane to give the trisphosphite intermediates. Oxidation at reduced temperature with *m*CPBA then gave the fully protected trisphosphates **52**–**55** respectively. ^{31}P NMR spectroscopy confirmed the presence of three phosphate groups in each compound.

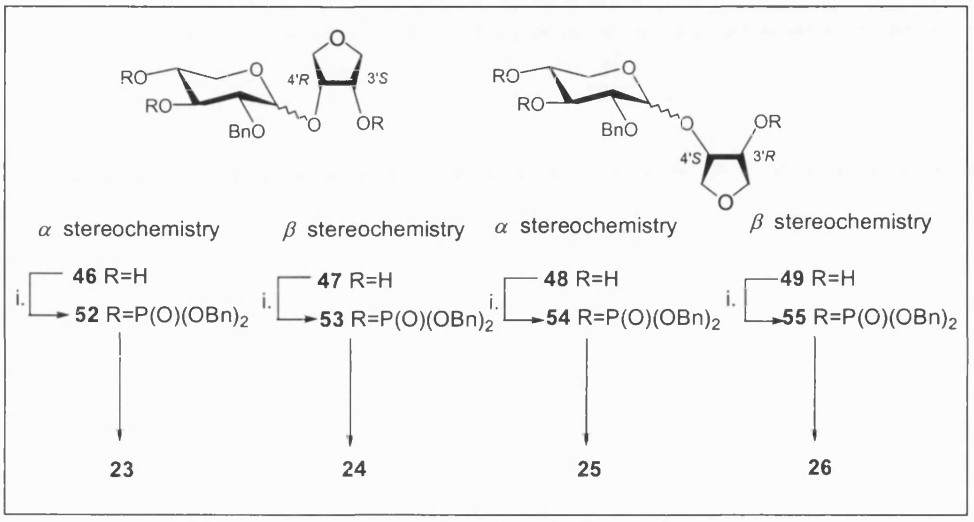


Figure 4.14: Phosphorylation and deprotection.

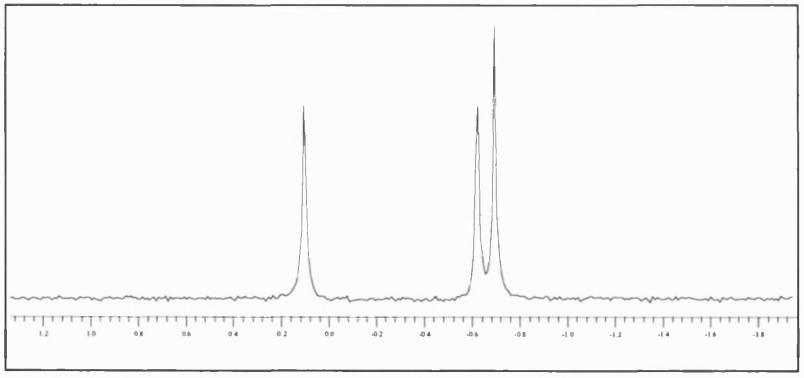


Figure 4.15: 162 MHz ^{31}P NMR spectrum of protected **23**.

Deprotection by hydrogenation over palladium on carbon yielded the target trisphosphates **23**–**26** respectively, which were purified by ion-exchange chromatography on Q Sepharose Fast Flow resin and isolated as their triethylammonium salts. Finally, each trisphosphate was accurately quantified by total phosphate assay.

The structures of the products were identified as the required triethylammonium salts of the trisphosphates (**23**–**26**) on the basis of their corresponding ^{31}P NMR and ^{1}H-

^{1}H COSY NMR spectra in CD_3OD. All the accurate negative FAB mass spectra showed a mass consistent with that predicted for $[M–H]^-$.

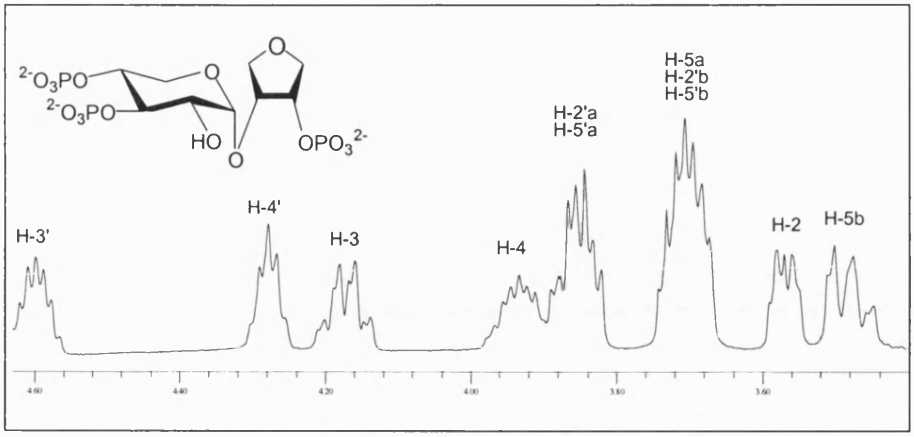


Figure 4.16: Part of 400 MHz 1H NMR spectrum of **23** in CD_3OD.

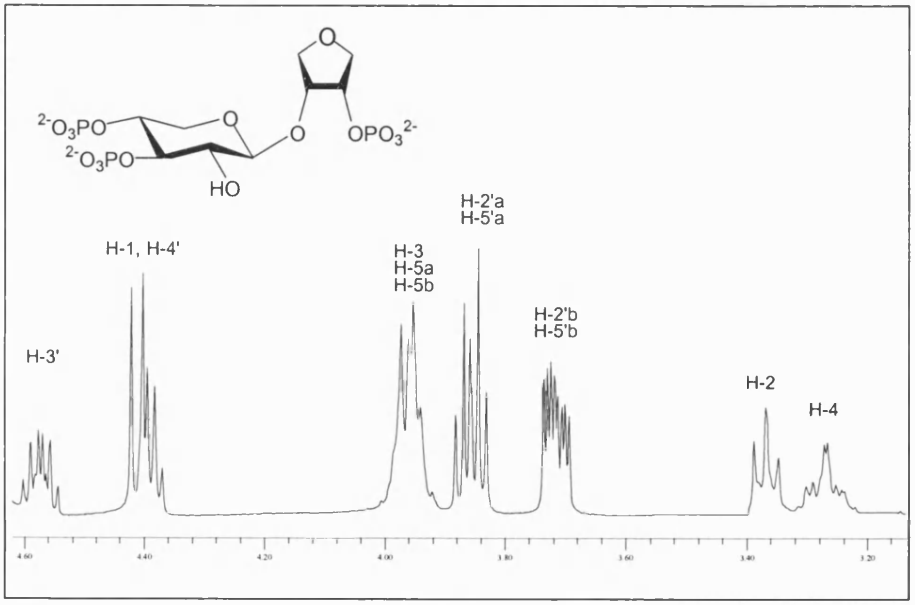


Figure 4.17: Part of 400 MHz ^{1}H NMRspectrum of **24** in CD_3OD.

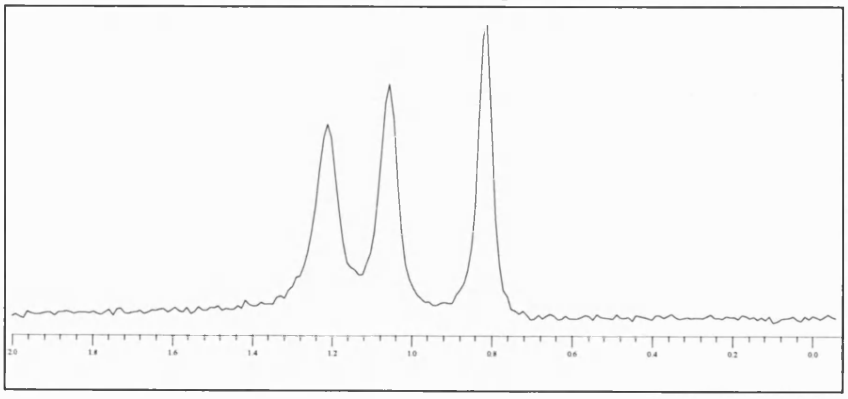


Figure 4.18: 162 MHz ^{31}P NMR spectrum of the triethylammonium salt of **26** in CD_3OD.

4.3 Biological Results

	Stereochemistry	EC_{50}	*h*	n	% release with 10 μm
$Ins(1,4,5)P_3$		144 ± 6 nM	1.68 ± 0.26	5	47 ± 2
23	*α*,3'*S*, 4'*R*	487 ± 58 nM	2.51 ± 1.00	3	51 ± 5
24	*β*,3'*S*, 4'*R*	nd	nd	5	3 ± 3
25	*α*,3'*R*, 4'*S*	nd	nd	5	12 ± 2
26	*β*,3'*R*, 4'*S*	2694 ± 276 nM	1.78 ± 0.43	3	46 ± 2

Table 4.1: $^{45}Ca^{2+}$ release data for $Ins(1,4,5)P_3$, **23**, **24**, **25** and **26** in permeabilised hepatocytes.
The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to logistic equations. Results are shown as means ± S.E.M.

Biological evaluation of the analogues by a collaborator using the method described in the experimental section indicated that maximally effective concentrations of $Ins(1,4,5)P_3$, **23** or **26,** released the same fraction of the intracellular Ca^{2+} stores (47 ± 2%, 51 ± 5% and 46 ± 2% respectively, Figure 4.18 and Table 4.1). Furthermore, the combined application of 10 μM $Ins(1,4,5)P_3$ with 10 μM of either **23** or **26** released no more Ca^{2+} than either of the agonists applied alone. The concentration of **23** required to cause half-maximal Ca^{2+} release (EC_{50}) was only 3–4 fold higher than that for $Ins(1,4,5)P_3$, while **26** was considerably weaker. The responses to both agonists were positively co-operative. Interestingly, there was no significant interaction between **24** (the *β*-epimer of **23**) and $Ins(1,4,5)P_3$ receptors; even at 10 μM **24** failed to cause significant Ca^{2+} mobilisation and nor did it affect the response to a submaximal concentration of $Ins(1,4,5)P_3$ (Table 2). Finally, the results for **25** are consistent with it being a very low affinity full agonist (EC_{50} >10μM): alone at 10 μM it released less than 50% of the $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores (Table 4.1) and its effects were approximately additive with a submaximal concentration of $Ins(1,4,5)P_3$ when added in combination with it (Table 4.2).

	% Ca^{2+} release	n
$Ins(1,4,5)P_3$ (150nM)	22 ± 4	3
24 (10μM)	4 ± 4	3
$Ins(1,4,5)P_3$ (150nM) with **24** (10μM)	30 ± 3	3
25 (10μM)	13 ± 3	3
$Ins(1,4,5)P_3$ (150nM) with **25** (10μM)	34 ± 1	3

Table 4.2: $^{45}Ca^{2+}$ release data for combined stimulation with $Ins(1,4,5)P_3$, **24** and **25** in permeabilised hepatocytes.
The percentage of the intracellular Ca^{2+} stores released by a submaximal concentration of $Ins(1,4,5)P_3$ alone or in combination with **24** and **25** are shown. Results are shown as means ± S.E.M for 3 independent experiments.

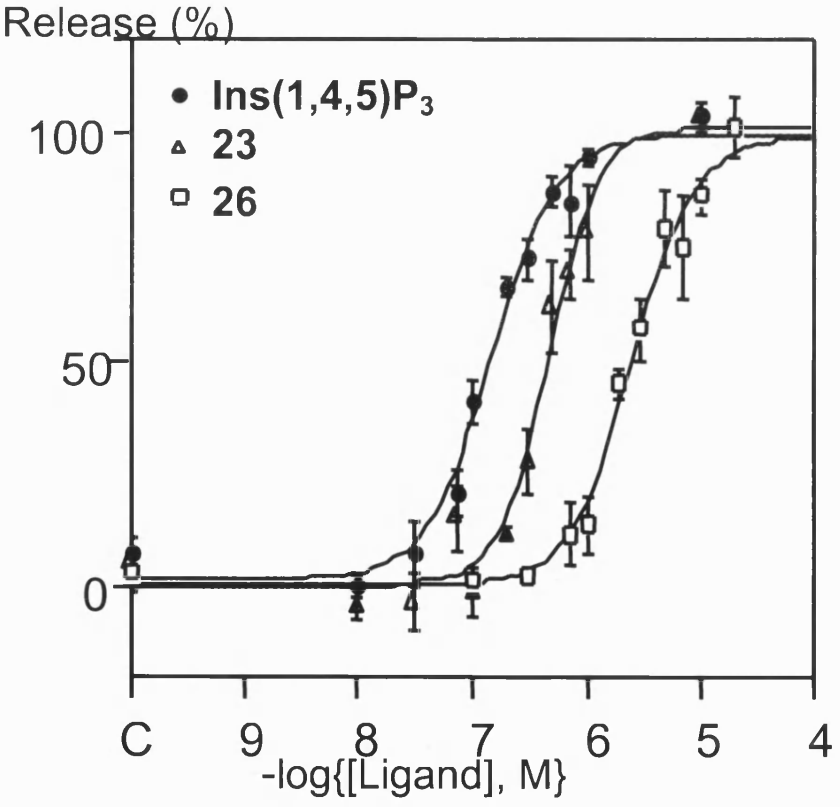


Figure 4.18: Effects of $Ins(1,4,5)P_3$ and xylopyranosides **23** and **26** on $^{45}Ca^{2+}$ release from the $Ins(1,4,5)P_3$-sensitive Ca^{2+} stores of permeabilised hepatocytes.

These results demonstrate that both **23** and **26** behave as full agonists in this assay and that diastereoisomer **23** has a potency in Ca^{2+} release assays similar to that of $Ins(1,4,5)P_3$, ribophostin and furanophostin. This shows that the 5-hydroxymethyl moiety of the glucose ring in ribophostin and furanophostin is not essential for potent

activity at Ins(1,4,5)P_3 receptors, although the very gradually decreasing potency in the series ribophostin>furanophostin>**23** suggests that each hydroxymethyl group in ribophostin may have a slight enhancing effect on activity. The results also confirm that, at the receptor binding site, the orientation of ribophostin and furanophostin relative to that of Ins(1,4,5)P_3 is such that the 5-hydroxymethyl group of the glucose moiety mimics the 3-hydroxyl of Ins(1,4,5)P_3. Had an inverted binding orientation been involved [in which case the 5-hydroxymethyl group would be equivalent to the 6-OH of Ins(1,4,5)P_3] then a dramatic difference in activity between the glucopyranoside-based analogues (ribophostin, furanophostin) and their xylopyranoside-equivalent (**23**) would have been expected, analogous to the large difference between Ins(1,4,5)P_3 and 6-deoxy-Ins(1,4,5)P_3. The fact that **23** (similar to ribophostin and furanophostin) is still 10 to 100–fold less potent than the adenophostins supports previous arguments that the adenine component of the adenophostins plays a pivotal role in their activity. The similar activities of Ins(1,4,5)P_3, ribophostin and furanophostin suggest that **23**, with its single hydroxyl group, is the minimal structure for potent agonism in a simple carbohydrate-derived agonist of Ins(1,4,5)P_3 receptors. Because this remaining OH group is equivalent to the crucial 6-OH of Ins(1,4,5)P_3, its removal would undoubtedly result in a dramatic decrease in potency. Indeed, while this work was in progress a report appeared [50] on a synthesis of the 2-deoxy derivative of **4.2**. This molecule, having no remaining hydroxyl group, was some 2000 fold less potent than Ins(1,4,5)P_3 in mobilising intracellular Ca^{2+}, showing the expected parallel with deletion of the 6-OH group of Ins(1,4,5)P_3[22].

4.4 Molecular modelling

Clearly, the stereochemical differences between analogues **23**, **24**, **25** and **26** have a significant effect on the recognition of these molecules by Ins(1,4,5)P_3 receptors. The structural simplicity of **23–26** suggested that a molecular modelling approach (which was carried out by Dr. A. M. Riley), might be used to account for the impact of stereochemical variation on their potencies. It is important to appreciate, however, that modelling of adenophostin A [48;62] and even of the simpler analogues **23–26** presents many difficulties, including those of simulating the combined influence of charged phosphate groups, solvent, counter-ions, pseudorotational equilibria in five-membered rings, and anomeric effects associated with the glycosidic linkage. It is also likely that

the conformation of these molecules is sensitive to the ionisation state [45;88] of the three phosphate groups under physiological conditions. With these reservations in mind, a consideration of predicted low-energy conformations of **23**–**26** can be used to suggest qualitative explanations for their differing potencies.

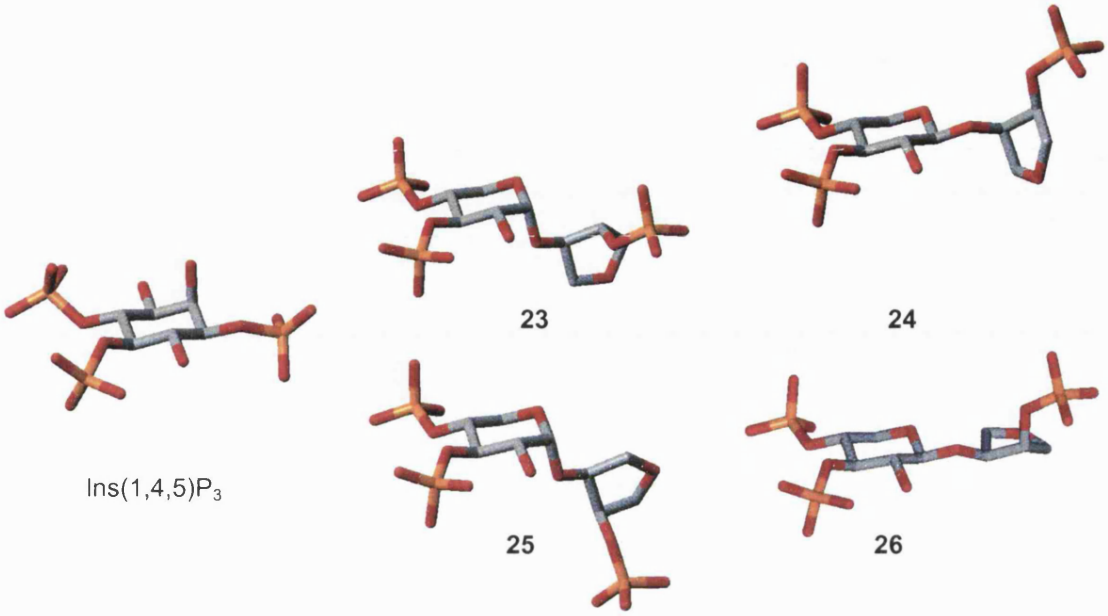


Figure 4.19: X-ray crystal structure of Ins(1,4,5)P_3 derived from its complex with the PH domain of phospholipase C-δ_1 compared with low energy conformers of diastereoisomers **23**–**26** as determined by molecular modelling experiments.

The most stable conformers found for **23**–**26** are shown in Figure 4.19, together with the conformation of Ins(1,4,5)P_3 identified in an X-ray crystallographic study of the PH domain of phospholipase C-δ_1 in complex with Ins(1,4,5)P_3 [37]. For diastereoisomer **23**, a particularly well-defined global minimum energy conformation was identified, similar to that reported for adenophostin A.* In this conformer, the tetrahydrofuranyl ring is positioned below the plane of the xylopyranosyl ring, presumably in an orientation that avoids steric clashes with the receptor binding pocket and does not disrupt binding of the 3′-phosphate group. This phosphate group is located in a similar region of space to the 1-phosphate group of Ins(1,4,5)P_3, although in a slightly more extended position. Thus, as has been suggested for adenophostin A

*It should be noted, however, that this conformation of **23** resembles a 3′-*endo* conformation of adenophostin A whereas, on the basis of ^{1}H NMR evidence, the 2′-*endo* form of adenophostin A is thought to predominate in solution. It has been suggested that the 2′-*endo* conformation might be essential to the high potency of adenophostin A [10].

[48;62;89], the non-vicinal phosphate in **23** may be held close to some ideal position for optimal interaction with the region of the receptor binding site that normally accommodates the 1-phosphate group of Ins(1,4,5)P_3. As in the case of adenophostin A [62] a local minimum conformer of **23** (corresponding to a 2′-*endo* conformation of adenophostin A) with slightly higher energy and a different puckering of the five-membered ring was also found.

In low energy conformations of the other diastereoisomers **24**–**26**, the 3′-phosphate group is held in different regions of space relative to the xylopyranosyl ring. Presumably the 3′-phosphate groups in the less active isomers **24**–**26** are, to different degrees, less able to interact effectively with the Ins(1,4,5)P_3 receptor binding pocket, and can only approach the ideal position and orientation at greater energetic costs than can the 3′-phosphate group in **23**. The almost negligible activity of **24** is striking, particularly when it is compared to that of the relatively potent β-linked analogue **26**. A possible explanation for this difference, in addition to the factors already discussed, is that in low energy conformations of **24** such as that shown in Figure 4.19, regions of the hydrophobic tetrahydrofuranyl ring may interfere with binding to the receptor.

4.5 Conclusions

The synthesis of a series of novel tetrahydrofuranyl xylopyranoside-based trisphosphates related to the adenophostins and to Ins(1,4,5)P_3 has been described. The most active member of the series (**23**) is of comparable potency to Ins(1,4,5)P_3 and to the previously described ribophostin and furanophostin, from which **23** is a logical development. Further simplification would inevitably result in a substantial decrease in potency, and analogue **23** is therefore likely to represent the simplest possible structure for potent Ca^{2+}-releasing activity in this type of carbohydrate-based analogue. The weaker activities of the other three diastereoisomers **24**, **25** and **26** may be related to the effect of differing stereochemistry at three stereogenic centres or the spatial positioning of the non-vicinal phosphate group and tetrahydrofuranyl ring.

4.6 Further xylose-based analogues

Several other xylose-based analogues have been reported since this work commenced, including *xylo*-adenophostin (figure 4.20), in which the glucose of adenophostin A has been replaced by xylose. *Xylo*-adenophostin was only 1.9 ± 0.6 fold less potent than adenophostin A, thus confirming our conclusions from $^{45}Ca^{2+}$ release results for xylofuranophostin.

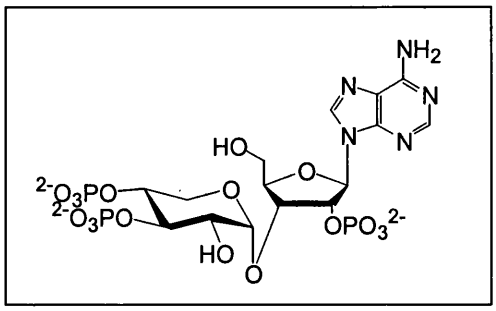


Figure 4.20: *Xylo*-adenophostin

Recently, two further xylose based analogues have been published which differ only in the stereochemistry at C-2 of the propyl tether between the xylose ring and adenine (figure 4.21). As would be expected from our results, the two compounds show strikingly different biological results. The effects of these analogues and Ins(1,4,5)P_3 on [^{3}H]Ins(1,4,5)P_3 binding and $^{45}Ca^{2+}$ mobilisation from rat hepatocytes which predominantly express type 2 Ins(1,4,5)P_3 receptors were recently compared. The data indicated that the order of potency was Ins(1,4,5)P_3>**4.6**>**4.7**. **4.6**, which has an absolute configuration at C-2' identical to adenophostin, has a much higher activity compared to **4.7.**

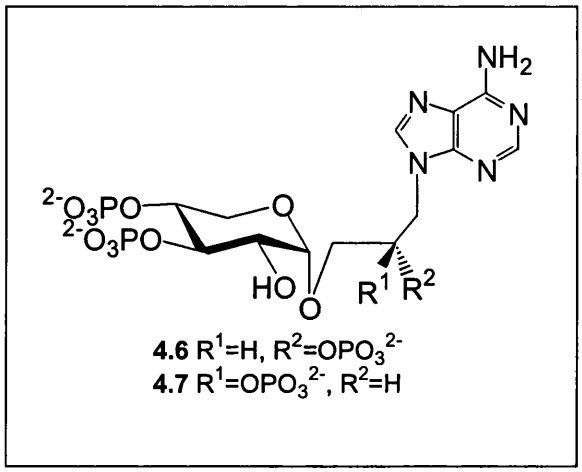


Figure 3.21: Further xylose based analogues.

Chapter Five

Synthesis of Adenophostin

and its

analogues.

Chapter 5

Synthesis of adenophostin A and its analogues.

5.1 Introduction

As previously discussed in chapters 3 and 4 the biological activity of analogues that lack the adenine base synthesised so far has not exceeded that of Ins(1,4,5)P_3. In an endeavour to ascertain the features of the adenine base crucial for adenophostin A-like activity, our group have recently reported the synthesis and biological results of several adenophostin A analogues where the nucleobase motif has been replaced by both natural and unnatural surrogates [43;65]. All the modified analogues were evaluated for Ca^{2+} mobilising activity in permeabilised hepatocytes relative to Ins(1,4,5)P_3 and adenophostin A.

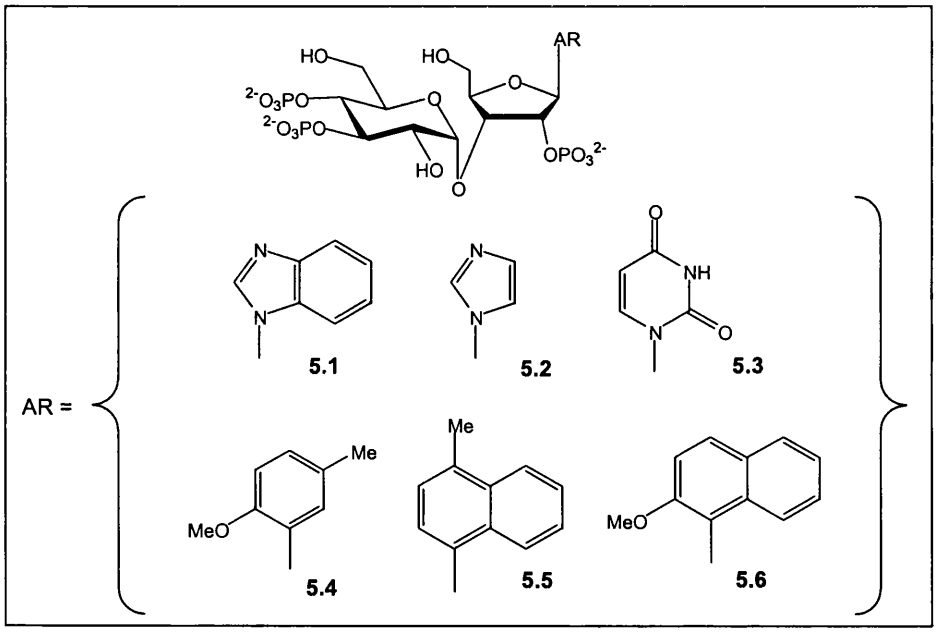


Figure 5.1: Base modified analogues

Replacement of the pyrimidine ring of the adenine by a benzene ring (**5.1**) reduced potency by approximately 4 fold, and complete removal reduced the potency of the analogue imidophostin (**5.2**) to almost that of Ins(1,4,5)P_3. Even replacement of the purine moiety (adenine) of adenophostin A with a much smaller pyrimidine (uracil **5.3**) caused the potency to decrease by approximately 2 fold. Finally, replacement of the entire adenine moiety of adenophostin A with unrelated single (**5.4**) or double (**5.5**, **5.6**) aromatic rings with a *β-C*-ribosyl glycosidic linkage

produced ligands with activities comparable to adenophostin A and greater than $Ins(1,4,5)P_3$.

Thus, all these compounds are more potent than $Ins(1,4,5)P_3$, and some have activity close to adenophostin A; however when the stereochemistry of the *C*-glycosidic linkage of **5.4** was changed to α from β the activity decreased dramatically to 10 times weaker than $Ins(1,4,5)P_3$.

It has now seems clear that in order to maintain the potency of adenophostin A an adenine base/base surrogate with a β-linkage to the ribose is required[43].

5.2 Synthesis of a versatile disaccharide building block

5.2.1 Discussion

The versatile disaccharide **56** (figure 5.2) has previously been utilised in the synthesis of adenophostin and its analogues. It is an excellent intermediate as it has orthogonal protecting groups. Furthermore, the acetates at positions one and two are a prerequisite for the Vorbrüggen condensation (figure 5.2). Several syntheses of this [58;59;64;65] and similar [61] disaccharides[71] have been published differing mainly in the glycosidation step and protecting group manipulations leading up to the glycosidation.

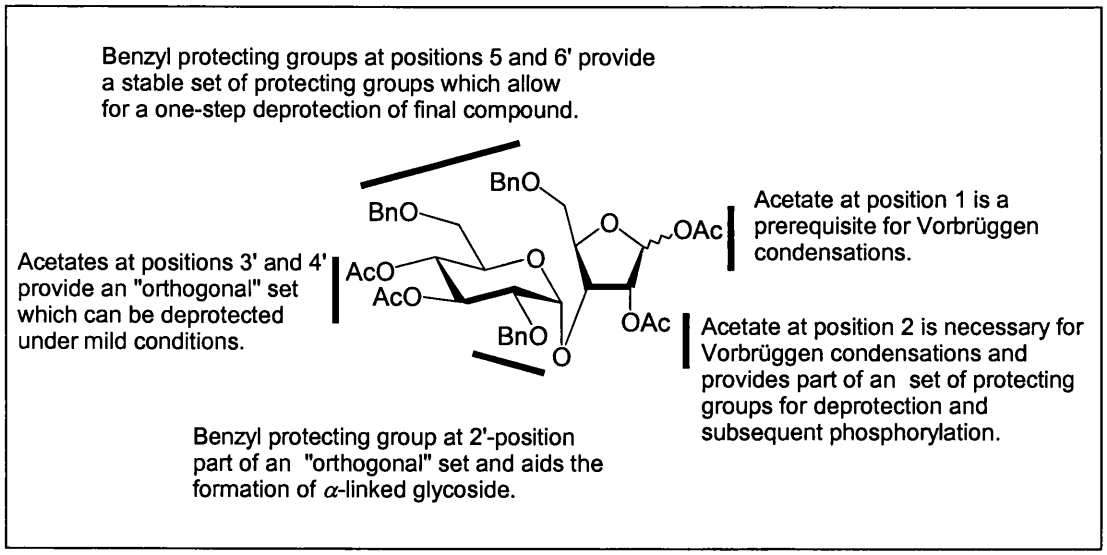


Figure 5.2: Versatility and synthetic utility of protected disaccharide donor (**56**).

In our laboratory we have previously utilised two different types of glycosidation procedure. Trichloroacetimidate methodology was used in the synthesis

of ribophostin[55], it was chosen as the literature reported high α selectivity in the glycosidation reaction[90]. Furthermore the trichloroacetimidate glycosyl donor could be prepared from a protected glucopyranose already in hand[46]. Unfortunately the desired β-glycoside was only obtained in moderate yield (48%), accompanied by a notable amount of the α-anomer. The glycosidation reaction proceeded smoothly to give predominantly the α-D-glucopyranosyl isomer however β-D-glucopyranosyl isomer constituted *ca.* 20% of the isolated product.

Phosphite methodology has been used in the synthesis of the disaccharide[64;65] and all the other analogues requiring glycosidation[57;63;91] including adenophostin[60]. It was chosen as model glycosidations using phosphite donors had been reported by Watanabe *et al*[92] with good α-selectivity and good yields. The phosphite donors were easy to prepare from the same glucopyranose used in the trichloroacetimidate route, and in quantitative yield.

Certain disadvantages of the phosphite methodology (e.g. preparation of the phosphite donor *in situ* each time) prompted us to explore an alternative strategy employing a thioglycoside donor. This approach has a number of attractive features; the thioglycoside offers sufficient temporary protection at the anomeric centre thus avoiding temporary protecting groups while the other hydroxyls are being protected[93]. Furthermore the donor is a crystalline solid rather than an oil which is a definite advantage when working with air sensitive reagents. The thioglycoside has relative stability to various reaction conditions and is conveniently activated with electrophilic reagents. Another attractive feature of thioglycosides as glycosyl donors is that they are readily converted into most other glycosyl donors. Finally, another group has recently reported the synthesis of the disaccharide using thioglycoside donors for the glycosidation which required fewer reaction steps than the synthesis of the phosphite donor[59].

5.2.2 Synthesis of the donor

The thiogylcosode donor was prepared on a large scale following the published procedures. Briefly, fully acetylated glucose was reacted with ethanethiol is the presence of a Lewis acid to produce the thiogylcoside **57**. The β-configuration of the thioglycoside was easily identifiable from the ^{1}H NMR spectrum, which

presented a doublet at δ_H 4.50 with a characteristically large coupling constant of J 9.9 Hz corresponding to H-1'. Longer reaction times led to an increase in α-configured product.

The selective 2,6 dipivaloylation methodology developed in chapter 3 to improve the yields of selectively protected donor was not attempted as there is evidence that in the absence of an adjacent axial function, the secondary 3-OH is more reactive than the 2-OH towards pivaloylation[73].

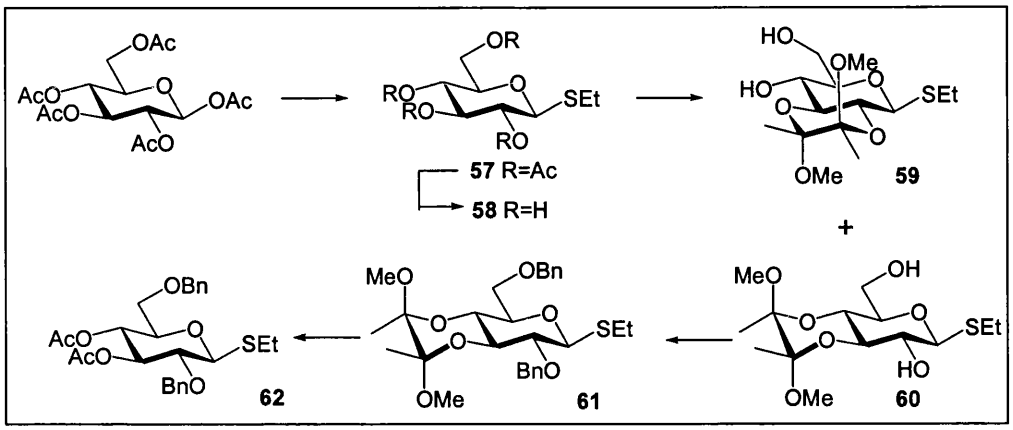


Figure 5.3: Route to the glycosyl donor **62**.

After the acetates were removed, the protection of the *trans*-diequatorial hydroxyl groups at positions 3 and 4 was achieved by refluxing **58** with butane-2,3-dione, catalytic camphorsulphonic acid and excess trimethyl orthoformate for 6 h. The two regioisomers were separated and the BDA derivative **60** was identified. The remaining 2 and 6 positions were alkylated with benzyl bromide and sodium hydride in DMF. This donor was initially used in the glycosidation step (see below), although it was later found to be more convenient to convert it into the 3,4-di-*O*-acetate. Thus cleavage of the BDA protection in **61** was accomplished by heating at reflux for a short time in a mixture of acetic acid and water. The mixture was concentrated and co-evaporated under reduced pressure with pyridine and then acetylated with an excess of acetic anhydride in pyridine to give the glycosyl donor **62**.

5.2.3 Synthesis of the acceptor

The desired acceptor was easily prepared from commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose following procedures already published[64]. The 5-

O-benzyl ether was synthesised in one step by the regioselective ring opening of a 3,5-*O*-dibutylstannylene derivative with benzyl bromide. An 87% yield of benzylated products was isolated containing 90% of the desired 5-*O*-benzyl regioisomer as indicated by ^{1}H NMR. Inversion of configuration at C-3to give the ribofuranoside acceptor was achieved by oxidation of this position with acetic anhydride and DMSO, followed by reduction of the 3-ulose intermediate with $NaBH_4$.

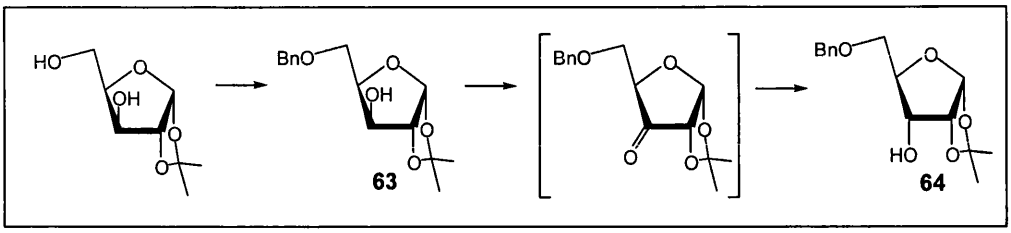


Figure 5.4: Synthesis of glycosyl acceptor

5.2.4 Glycosidation

Glycosidation of ribose acceptor (**64**) with glucose donor (**62**) in the presence of the promoter NIS and catalytic amount of triflic acid proceeded in a stereoselective fashion to afford α-linked disaccharide in 68 % yield. Formation of the required 1,2 *cis* linkage was confirmed by the H-1" resonance in the ^{1}H NMR spectrum, which appeared as a deshielded doublet at δ_H 5.82 with a characteristically axial-equatorial coupling constant of J 3.9 Hz. Glycosidation of the ribose acceptor (**64**) and glucose donor (**61**) was also carried out to reduce the number of steps in the synthesis; although the reaction was successful the yield was slightly lower. Furthermore the glycosidation of the acceptor with the acetylated donor also has the advantage of producing a crystalline product.

Cleavage of the *cis*-isopropylidene acetal was accomplished by stirring with TFA for 10 min at rt to yield the corresponding *cis*-diol (**66**). The 1,2-di-*O*-acetate was then prepared by treatment with acetic anhydride, in order to meet the Vorbrüggen condensation criteria of acyl groups at these positions[65].

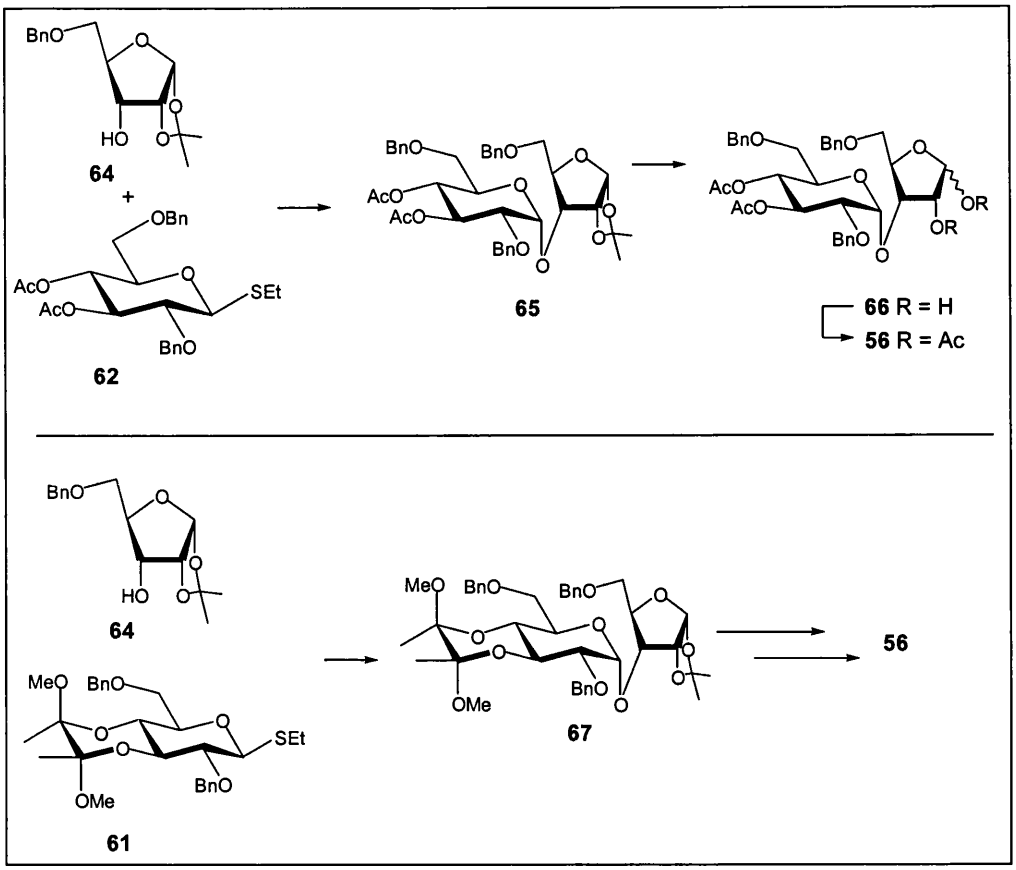


Figure 5.5: Two routes to the disaccharide intermediate (**56**).

5.3 Synthesis of adenophostin A

5.3.1 Vorbrüggen condensation and deprotection

Synthesis of nucleosides *via* the reaction of silylated heterocyclic bases with peracetylated sugars in the presence of Friedel-Crafts catalysts is now well established. Vorbrüggen *et al.* first used TMSOTf as the catalyst 1981[94]; previously $SnCl_4$ had been used, but it was difficult to remove in the work up. TMSOTf was found to be a better catalyst because it was easily removed in the work up and gave higher yields.

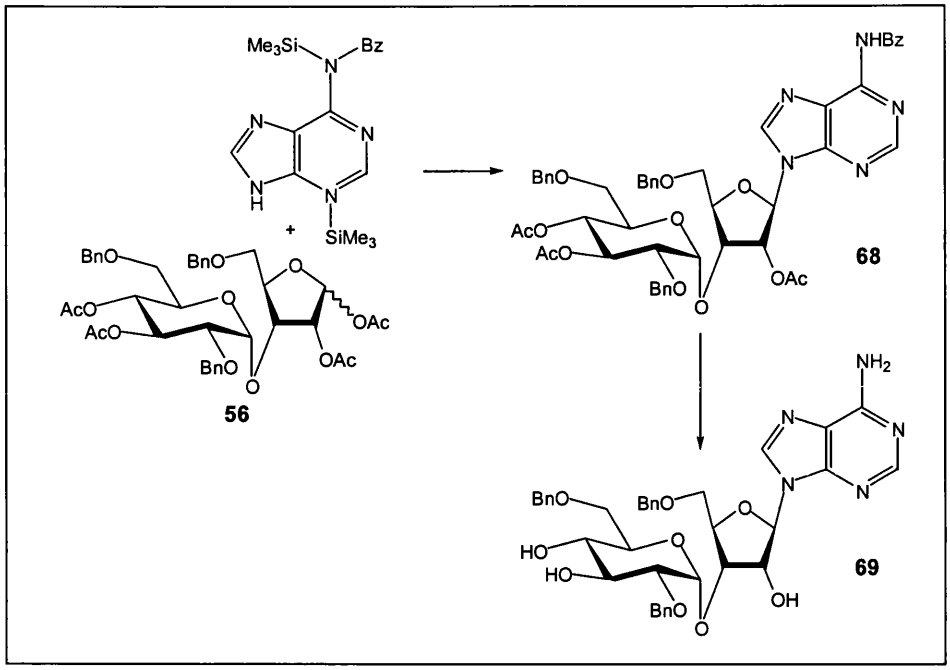


Figure 5.6: Route to triol **69**.

Vorbrüggen condensation reaction of disaccharide **56** with silylated N-6-benzoyl-adenine in the presence of catalytic TMSOTf proceeded smoothly. TLC during the course of the reaction revealed the formation of two products. Initially the lower spot was the major one, but over time saw full conversion to the higher. The compound was identified by its ^{1}H NMR spectrum which exhibited a deshielded doublet at 6.42 ppm with a characteristically axial-axial coupling constant of J 5.5 Hz corresponding to H-1' of a β-substituted product (**68**).

The acetyl groups and benzoyl group were simultaneously removed with excess lithium hydroxide in water/methanol overnight yielding the corresponding triol (**69**) which was purified by flash chromatography.

5.3.2 Phosphorylation and deprotection

Selective phosphitylation of the free hydroxyls without the prior protection of the adenine N-6-position, was then attempted by the published procedure with a mixture of bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate in DCM[60;63]. Monitoring the reaction by TLC indicated that the reaction never went to completion and some over-phosphitylation occurred. The reaction mixture was cooled to –78°C before being oxidised with *m*CPBA. The reaction was quenched at

this temperature to avoid any possible oxidation of the adenine base. The yield was low as the expected product of this reaction (**71**) was extremely difficult to isolate.

A review of the literature found that the original adenophostin synthesis by Hotoda *et al*[42] made use of 4,4'-dimethoxytritylation (DMTr) protection at the amino group of the adenine and the two primary hydroxyl groups. Since the primary hydroxyls are protected in **69**, only the amino group of the adenine needed protecting in this instance. Thus **69** was dissolved in dry pyridine and subsequent addition of 4,4'-dimethoxytrityl chloride resulted in the introduction of a DMTr group at the N-6 of adenine.

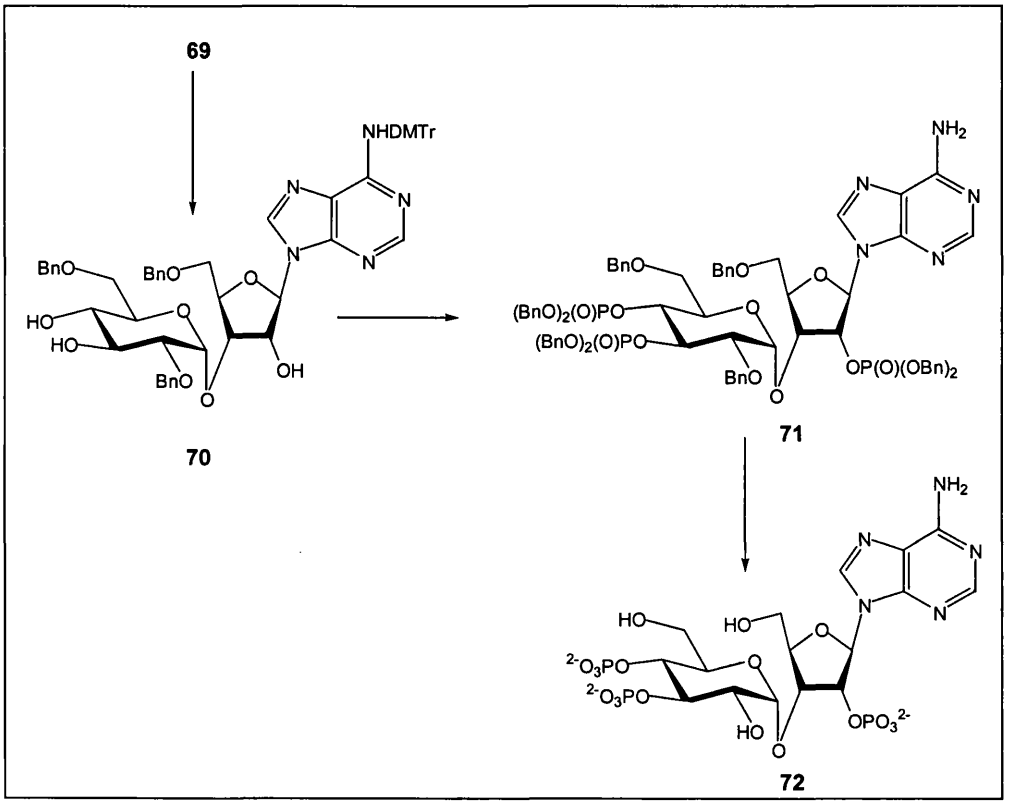


Figure 5.7: Phosphitylation and deprotection.

Subsequent phosphitylation of the triol with 1*H*-tetrazole and bis(benzyloxy)(diisopropylamino)phosphine in dichloromethane gave the trisphosphite. The reaction mixture was cooled to –78°C and oxidised with *m*CPBA, to give the fully protected trisphosphate, which was immediately deprotected with acetic acid to give **71** in 85% yield. ^{31}P NMR spectrum of the purified product (**71**) confirmed the presence of three phosphate groups. In addition, the presence of the free unphosphorylated N-6-position NH_2 was substantiated by the presence of a broad singlet in the ^{1}H NMR at δ_H 5.81.

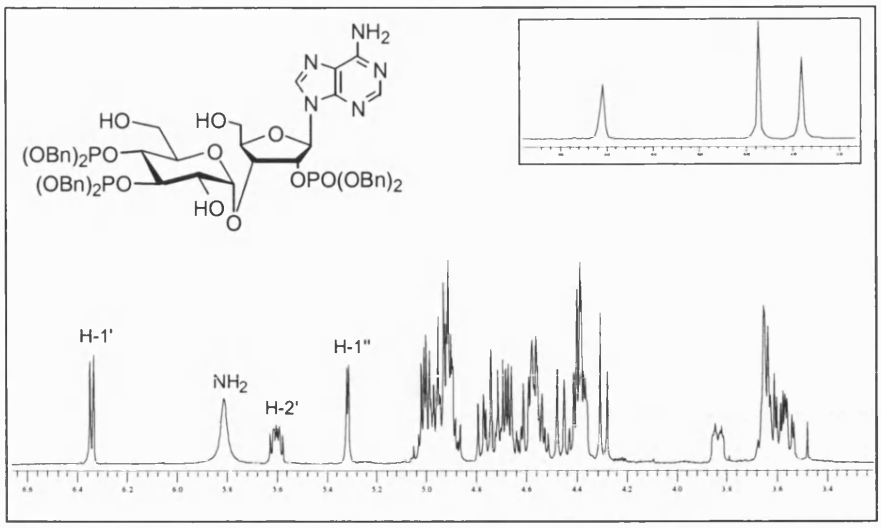


Figure 5.8: Part of the ^{1}H NMR spectrum of **71** clearly indicating the free N^{6}-amine of adenine and part of the ^{31}P NMR (insert) indicating three protected phosphate groups.

Deprotection of this adenophostin precursor was achieved via catalytic transfer hydrogenation[60;63]. A solution of **71** was refluxed in a mixture of methanol, water and cyclohexene with 20% palladium hydroxide for 3 h. The free acid was then converted into the sodium salt. ^{31}P NMR exhibited three resonances from the three deprotected phosphates and the ^{1}H NMR spectrum of adenophostin A was in keeping with the published data[42;60;61].

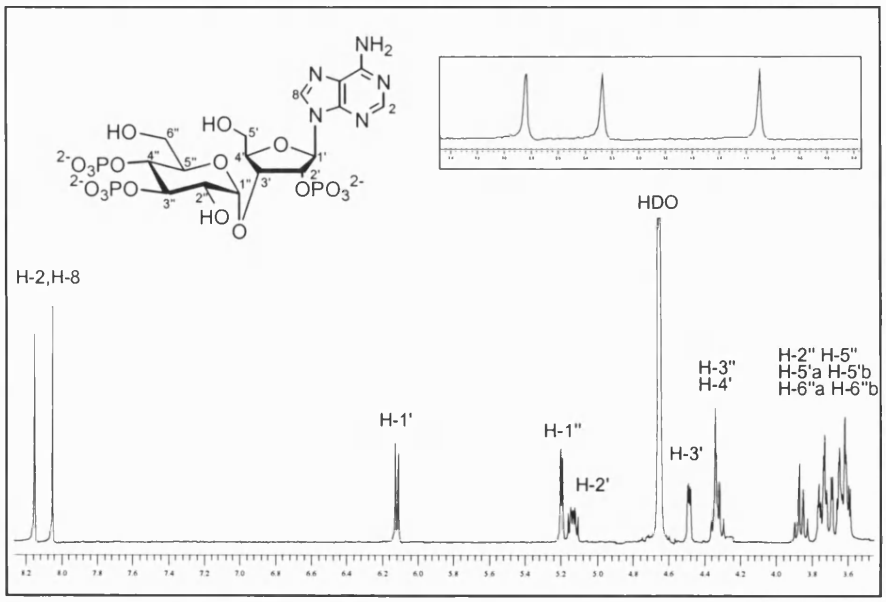


Figure 5.9: 400MHz ^{1}H NMR spectrum of the free acid of adenophostin A in D_2O and part of the ^{31}P NMR (insert) indicating three protected phosphate groups.

5.4 N-6 Base-Modified Adenophostins

5.4.1 Introduction

In order to explore the base-modification approach further we decided to synthesis a series of N-6 base modified compounds for the following reasons. The most active totally synthetic adenophostin A analogue so far published is purinophostin[64] where the amino group has simply been removed from the adenine; this suggested that, although a purine ring (or equivalent) is necessary for high potency, the 6-amino constituent does not contribute greatly to the activity of adenophostin A. Furthermore, previous results for the activity of the hypoxanthine analogue[42], prepared by the deamination of natural material, indicated that it too had activity similar to that of adenophostin A.

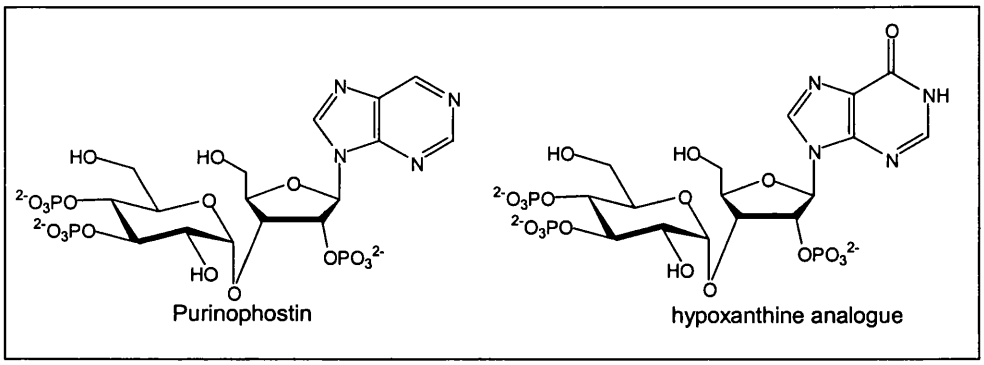


Figure 5.10: Literature N-6 substituted analogues.

The N-6 base-modified analogues are elaborated versions of adenophostin A designed to explore predominately the introduction of a range of different sized hydrophobes. The synthesis of these modified analogues is versatile and is an extension of the general methodology for synthesising base substituted compounds.

In the literature there are many examples of N-6 substituted analogues of adenosine; most of the syntheses described begin with 6-chloropurine as the heterocyclic base to gain easy access to any kind of N-6 monosubstituted adenosine analogue[95] and references therein. Substituting the benzoyl-adenine with 6-chloropurine in the Vorbrüggen reaction with the disaccharide led to a versatile adenophostin intermediate capable of undergoing nucleophilic substitution with a range of nucleophiles. From this intermediate a variety of novel analogues has been

synthesised. It may also be possible to synthesise adenophostin by substituting the chlorine with ammonia, and purinophostin via the removal of the chlorine by hydrogenolysis.

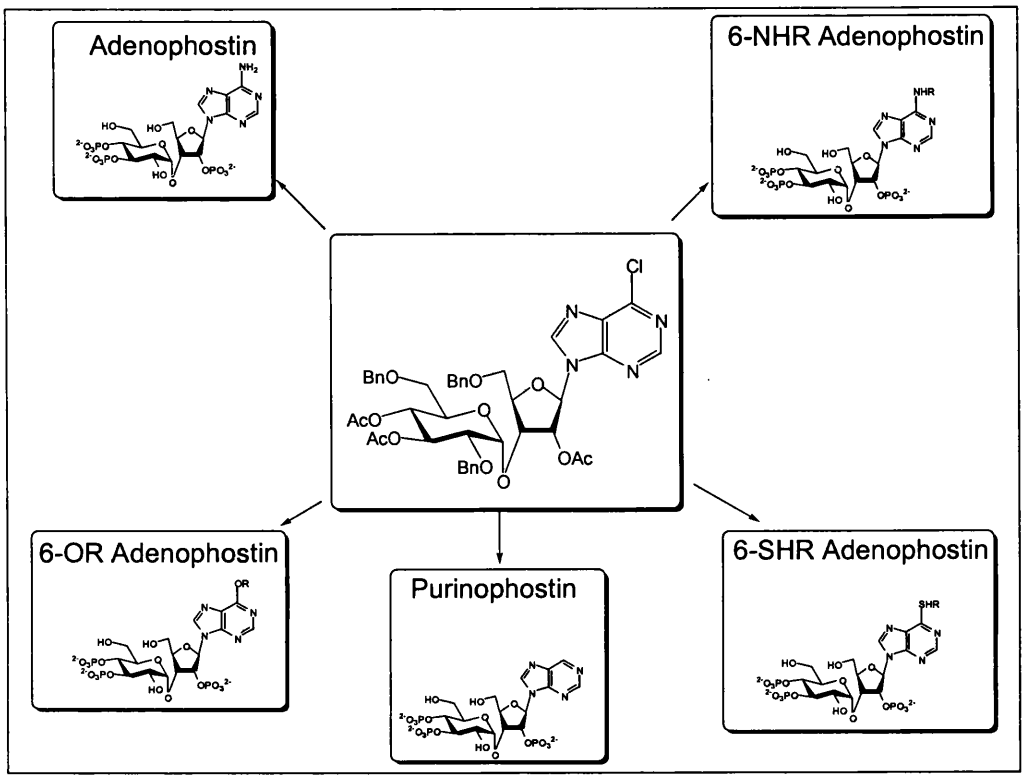
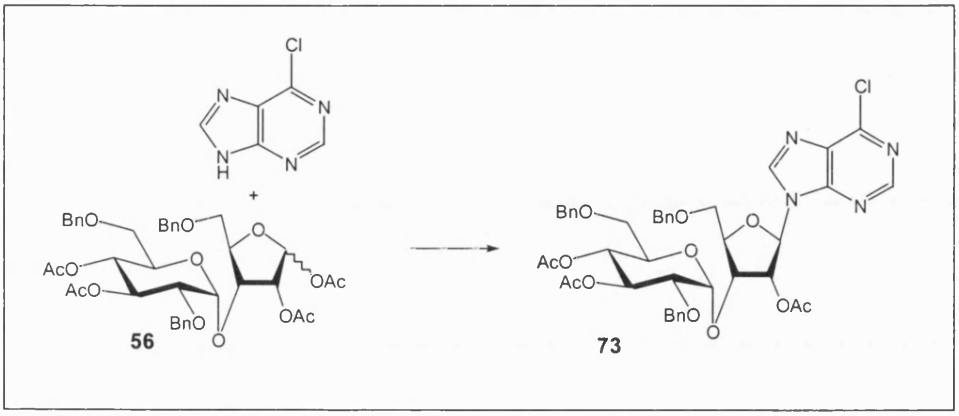


Figure 5.11: potential products from substitution reactions.

5.4.2 Vorbrüggen Condensation

Condensation of dissacharide (**56**) and 6-chloropurine yielded the key intermediate 2',3",4"-tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-6-chloro-1-β-D-ribofuranosidepurine (**73**). For the condensation reaction a modified Vorbrüggen reaction was used[96]. The mixture of protected disaccharide and base was treated with TMSOTf and DBU in acetonitrile at 60 °C for 1 h. DBU is used not only as a nucleophilic base but it increases the solubility of 6-chloropurine. TMSOTF acts as a catalyst to convert the 1,2-di-*O*-acyl ribofuranoside into an electrophilic 1,2-acyloxonium salt. As an excess of TMSOTf was used, the meticulous drying of reagents and solvents necessary for the success of the Vorbrüggen coupling could be dispensed with. The product was isolated in excellent yield (89%) by flash chromatography and identified by its ^{1}H NMR spectrum which exhibited a deshielded

doublet at 6.41 ppm corresponding to H-1' of a β-substituted product. Finally, the positive ion FAB mass spectrum for this compound exhibited a peak at *m/z* 846 corresponding to the compound, and showed a characteristic isotope pattern for chlorine.



Scheme 5.1: Synthetic route to versatile 6-chloro intermediate (**73**).

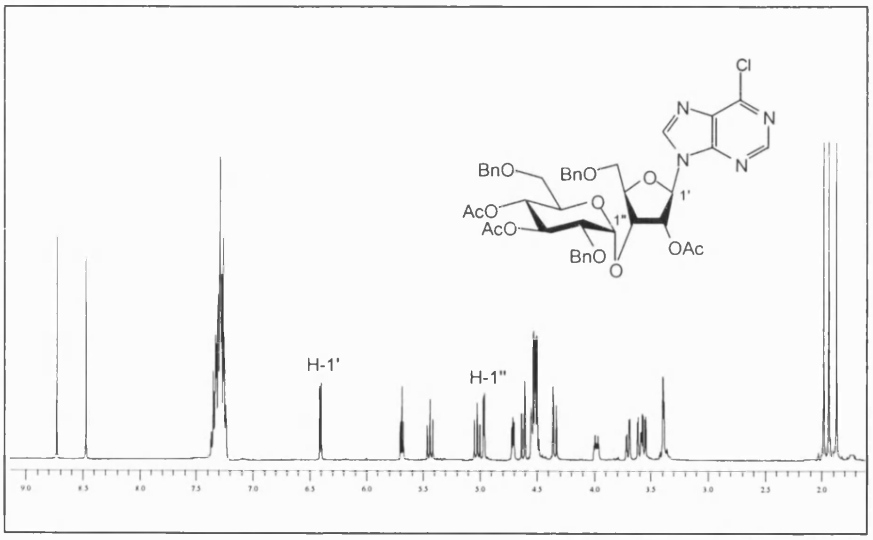


Figure 5.12: 400 MHz ^{1}H NMR spectrum of **73** in $CDCl_3$ indicating the anomeric positions.

5.5 Synthesis of 6-OMe adenophostin

With the versatile 6-chloro intermediate (**73**) in hand attention turned to the first substituted product. The 6-methoxy compound was attempted first, as the literature revealed that NaOMe[97] was used for the synthesis of the adenosine equivalent 6-methoxy analogue from the known 6-chloropurine riboside. Since

catalytic NaOMe is known to remove acetate protecting groups no prior deprotection was required.

The 6-chloro derivative (**73**) was easily converted into the 6-methoxy triol. Thus **73** was treated with two equivalents of NaOMe for 30 min at 65 °C to give after purification by flash chromatography **74** in 74% yield.

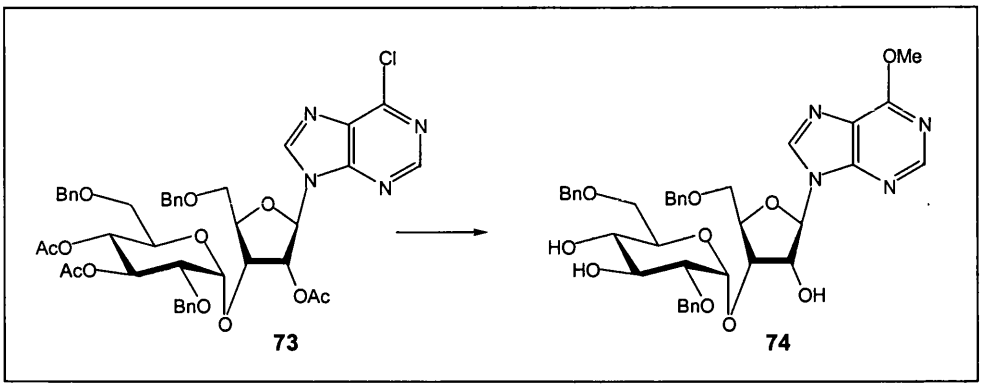


Figure 5.13: 6-methoxy triol **74**.

5.5.1 Phosphorylation and deprotection

Triol **74** was reacted with a pre-formed complex between 1*H*-tetrazole and bis(benzyloxy)(diisopropylamino)phosphine in dichloromethane. The resulting trisphosphite was oxidised with *m*CPBA to the trisphosphate. The ^{31}P NMR spectrum of the purified product exhibited three singlets corresponding to the three dibenzyloxy phosphate groups.

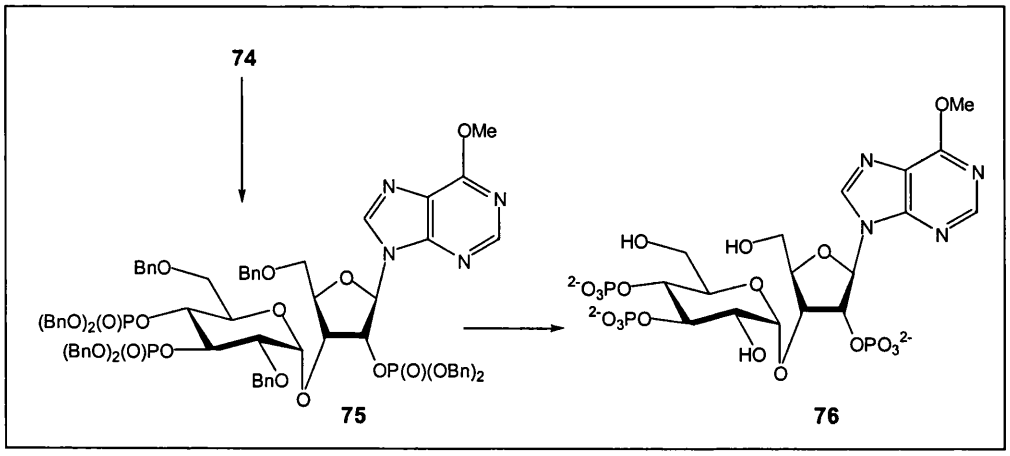


Figure 5.14:

Deprotection of **75** to give the methoxy analogue of adenophostin A was achieved with catalytic transfer hydrogenation in a similar manner to adenophostin. However complete deprotection took 31 hours compared to 3 hours needed to deprotect adenophostin and the corresponding N-substituted analogue precursors.

This is possibly due to the electron donating effects of the methoxy group or to coordination of the oxygen with the palladium. The free acid was then converted into the sodium salt. ^{1}H NMR of the white solid product indicated complete loss of all the benzyl groups, and ^{31}P NMR exhibited three resonances from the three deprotected phosphates. Quantification was carried out with the Briggs phosphate assay.

5.6 Synthesis of N6-cyclopentyl-adenophostin

The majority of the literature procedures for synthesising N6-substituted adenosine analogues have used the commercially available 6-chloropurine riboside as the starting material. It was therefore decided that the base labile acetates of **73** should be removed prior to the nucleophilic displacement of the chlorine with the cyclopentylamine. Thus the base labile acetates of **73** were removed using catalytic NaOMe in methanol. Unfortunately a side product was produced which, not surprisingly, was identified as the 6-methoxide substituted triol **74** on the basis of TLC with an authentic sample of **74** and ^{1}H NMR. The 6-chloro triol was then heated with cyclopentylamine and triethylamine at 80 °C for 3 hours. This reaction proceeded smoothly to produce isolated **78** in 54% yield after purification by flash chromatography.

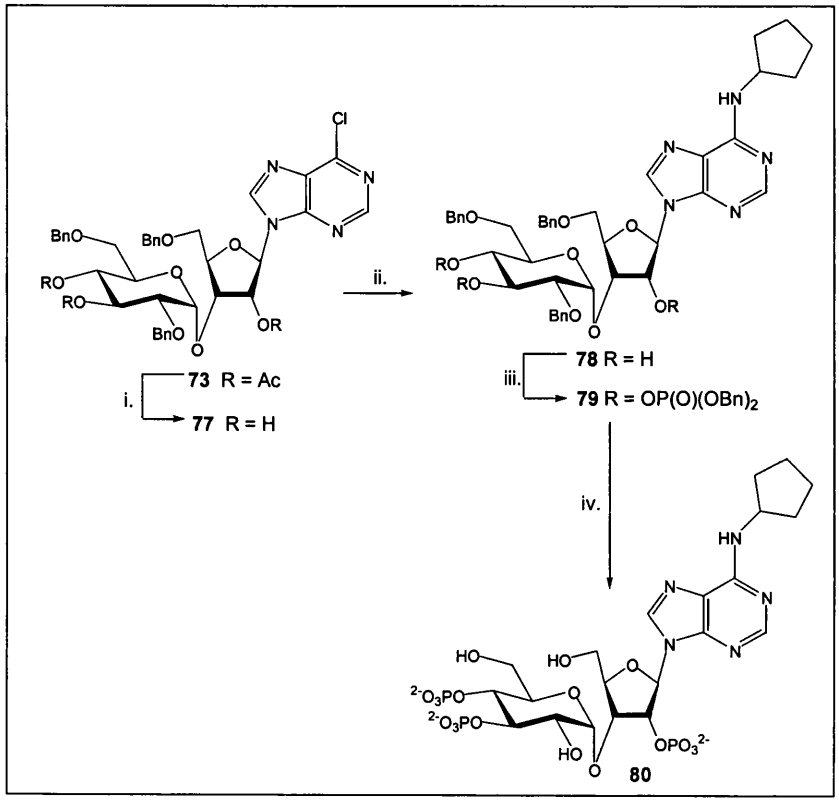


Figure 5.15: Route to N6-cyclopentyl-adenophostin **80**.

5.6.1 Phosphorylation and deprotection

Triol **77** was phosphitylated with a pre-formed complex between 1*H*-tetrazole and bis(benzyloxy)(diisopropylamino)phosphine in dichloromethane. The resulting trisphosphite was oxidised with *m*CPBA at –78°C to give the trisphosphate. The ^{31}P NMR spectrum of the purified product exhibited three singlets corresponding to the three dibenzyloxyphosphate groups at 0.15, –0.66 and –0.79ppm.

Complete deprotection was achieved with catalytic transfer hydrogenation. **79** Was heated at 65 °C in a mixture of cyclohexene, methanol and water with 20% palladium hydroxide on carbon for 3 hours. After removal of the catalyst by filtration the product was converted to its sodium salt. The structure of the product was identified as the sodium salt of the trisphosphate **80** on the basis of its ^{31}P NMR spectrum which showed three signals at 3.28, 2.56 and 1.59 ppm, and by its ^{1}H NMR in D_2O indicated complete loss of benzyl groups. The accurate negative ion FAB mass spectrum showed a mass consistent with that predicted for $[M–H]^-$. The compound was quantified by Briggs phosphate assay.

5.7 Synthesis of N-6 methyl, dimethyl and cyclohexyl-adenophostins

Due to the previous problems of the acetate deprotection in the presence of the chlorine (see cyclopentyl) it was decided that it would be better to substitute the chlorine first. The first compound attempted was the N-6 methyl-substituted compound **85**. Thus **73** was heated with triethylamine and methylamine hydrochloride at 60 °C overnight. Initially using 3 equivalents of methylamine hydrochloride and 6 equivalents of triethylamine, the product could be isolated, although the reaction did not go to completion. The structure of **81** was established on the basis of its ^{1}H NMR spectrum in $CDCl_3$ which showed the appearance of an NH signal at 5.87 ppm and NCH_3 signal at 3.19 ppm. When the equivalents were raised to 7 and 14 respectively, all starting material disappeared but an inseparable side product appeared. As the reactants are fairly nucleophilic it was presumed that the side product formed was a partially deprotected product, therefore the mixed products were treated with catalytic amount of NaOMe to produce only the desired product.

Substitution by the dimethylamine went to completion within 2 h and no deprotection occurred. However substitution with cyclohexylamine required longer reaction times and some acetate deprotection occurred. The products were fully deprotected using catalytic amount of NaOMe in methanol to give the triols **87** and **91** which were purified by crystallisation.

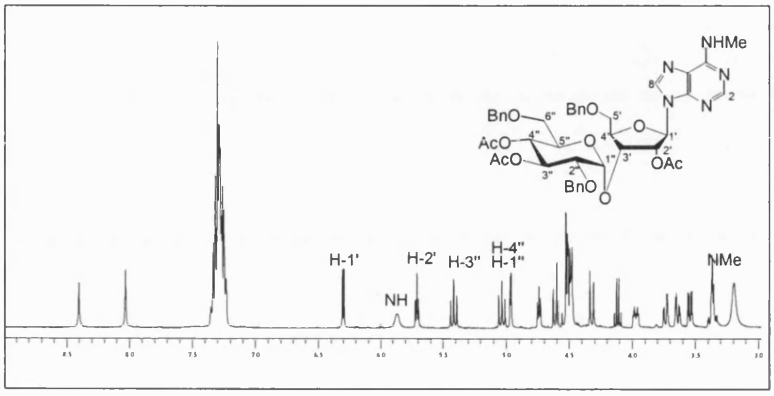


Figure 5.16: Part of the ^{1}H NMR spectrum of **81** clearly indicating the NHMe, the anomeric protons and the acetylated positions.

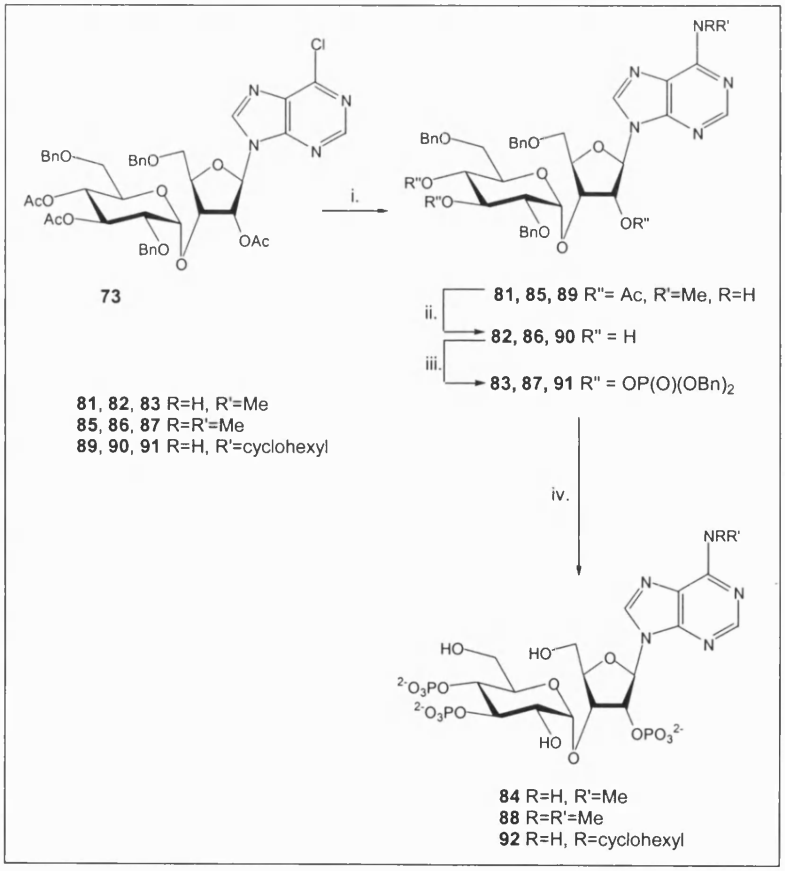


Figure 5.17: Route to trisphosphates

5.7.1 Phosphorylation and deprotection

The triols **82**, **86**, **90** were phosphitylated in the usual way with a pre-formed complex between 1*H*-tetrazole and bis(benzyloxy)(diisopropylamino)phosphine in dichloromethane. The trisphosphites were oxidised with *m*CPBA at –78°C to give the fully protected intermediates. The ^{31}P NMR spectrum of the purified products exhibited three singlets corresponding to the three dibenzyloxy phosphate groups. ^{1}H coupled ^{31}P NMR spectrum of **83** in CDCl3 exhibited the protected ring phosphates as a sextet at –0.23 ppm and two overlapping sextet at –1.05 ppm.

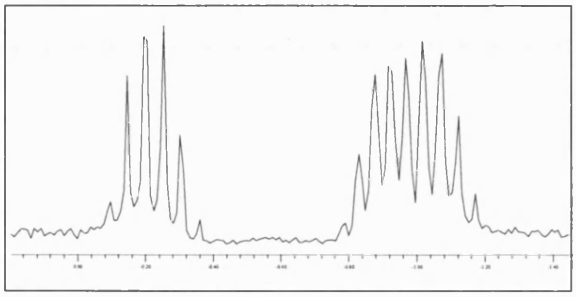


Figure 5.18: 162MHz ^{1}H-coupled ^{31}P NMR spectrum of **83** in $CDCl_3$.

Unfortunately, the dimethyl compound (**86**) contained some impurities which could not be entirely removed by column chromatography with various eluents. As the impurities did not contain phosphorus, as judged by ^{31}P NMR spectroscopy, the crude material was deprotected. The target trisphosphate was obtained pure after HPLC.

Deprotection of all three compounds was achieved with catalytic transfer hydrogenation as described for the synthesis of adenophostin. The compounds were quantified by Briggs phosphate assay, samples were examined by HPLC and the resulting HPLC traces are shown below in Figure **5.19**.

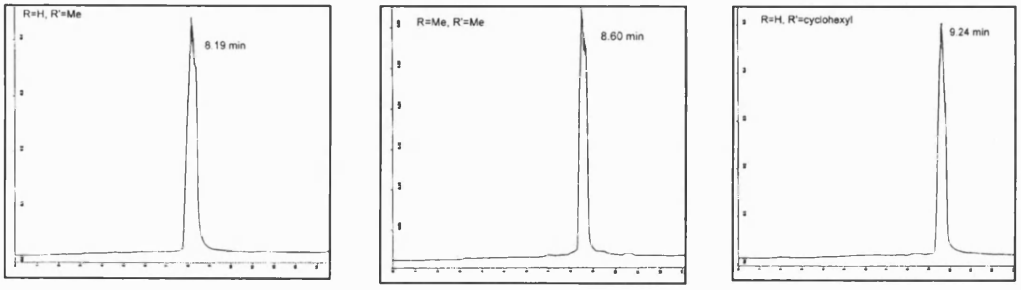


Figure 5.19: HPLC traces of **84**, **88** and **92**.

5.8 Synthesis of N-6 methyl-2-OMe-adenophostin

In order to elaborate the adenine moiety further a synthesis of a 2,6 disubstituted adenophostin analogue was attempted. In the literature there are many examples of N-6, N-2 disubstituted analogues of adenosine; starting from 2,6-dichloropurine as the heterocyclic base to gain access to many analogues. Furthermore it has been demonstrated that the 6-chlorine atom is much more reactive than the 2-chlorine atom in the adenosine derivative; it is logical to presume that the chlorine atoms in the corresponding adenophostin derivative should show a similar difference in reactivity[97]. Thus, monosubstitution of the 2,6 dichloropurine derivative by a nucleophilic reagent should yield a 6-substituted product, which after isolation, should be capable of undergoing further substitution of the 2-position with a variety of nucleophilic reagents.

5.8.1 Vorbrüggen Condensation

The method employed to prepare 2',3",4"-tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-2,6-dichloro-1-β-D-ribofuranosidepurine (**93**) is similar to that used in the synthesis of **73**. and involves a modified Vorbrüggen reaction. Thus a mixture of protected disaccharide and 2,6-dichlorpurine was treated with TMSOTf and DBU in acetonitrile at 60 °C for 1 h. The reaction was difficult to follow as there was no change in Rf value from the starting material to product when TLC was carried out in a variety of solvents; however, the product was isolated in good yield (67%) by flash chromatography and identified by ^{1}H NMR spectrum which exhibited a deshielded doublet at 6.37 ppm corresponding to H-1' of a β-substituted product and a singlet at 8.45 ppm corresponding to H-8.

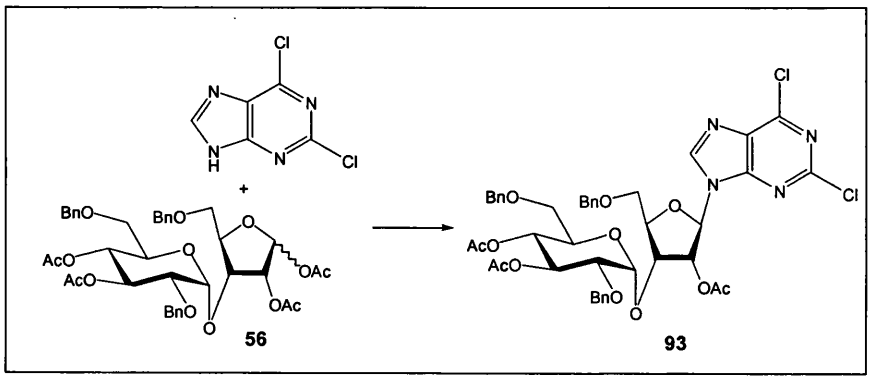


Figure 5.20: Route to versatile 2,6 dichloro-intermediate **93.**

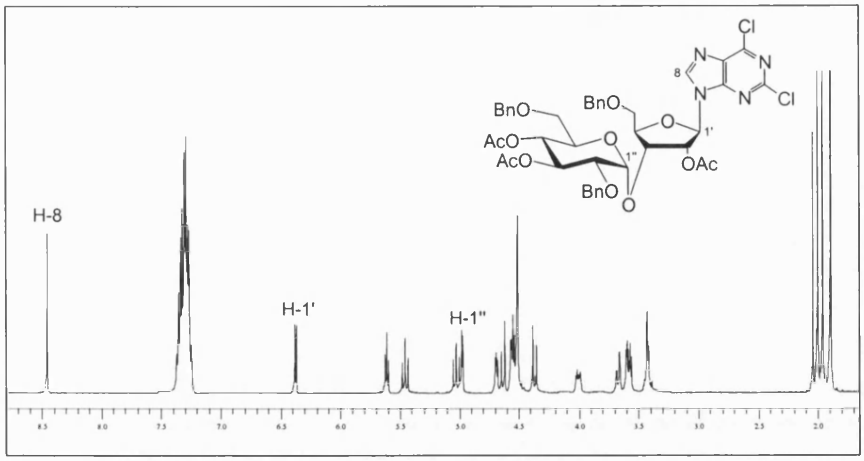


Figure 5.21: 400 MHz ^{1}H NMR spectrum of **93** in $CDCl_3$ indicating the anomeric positions and H-8.

5.8.2 Substitution reactions

With the 2,6-dichloro-derivative in hand attention then turned to substitution reactions. Initially the 2,6-dimethyl compound was attempted. Thus, **93** was refluxed with a large excess of methylamine hydrochloride and triethylamine in dichloromethane and ethanol overnight. TLC showed all starting material had disappeared and the formation of several inseparable products. The crude material was subjected to FAB mass spectrometry unfortunately there was no peak corresponding to the disubstituted product. The reaction time was reduced to 4 h and one major product was isolated and identified as 6-NMe, 2-chloro derivative on the basis of its ^{1}H NMR and FAB mass spectrum.

It was then decided that 2-methoxy substitution would be attempted with the simultaneous removal of the acetate protecting groups. Thus 2 equivalents of NaOMe were added to a solution of **94** in methanol and heated at 65 ^{o}C overnight. The product was identified as **95** by ^{1}H NMR and FAB mass spectrometry which still exhibited a characteristic isotope pattern for chlorine. It was therefore reasoned that more forcing conditions were required for substitution. Thus **95** was refluxed with a very large excess of NaOMe in methanol overnight. The product was identified as the required triol (**96**) after purification by flash chromatography and crystallisation.

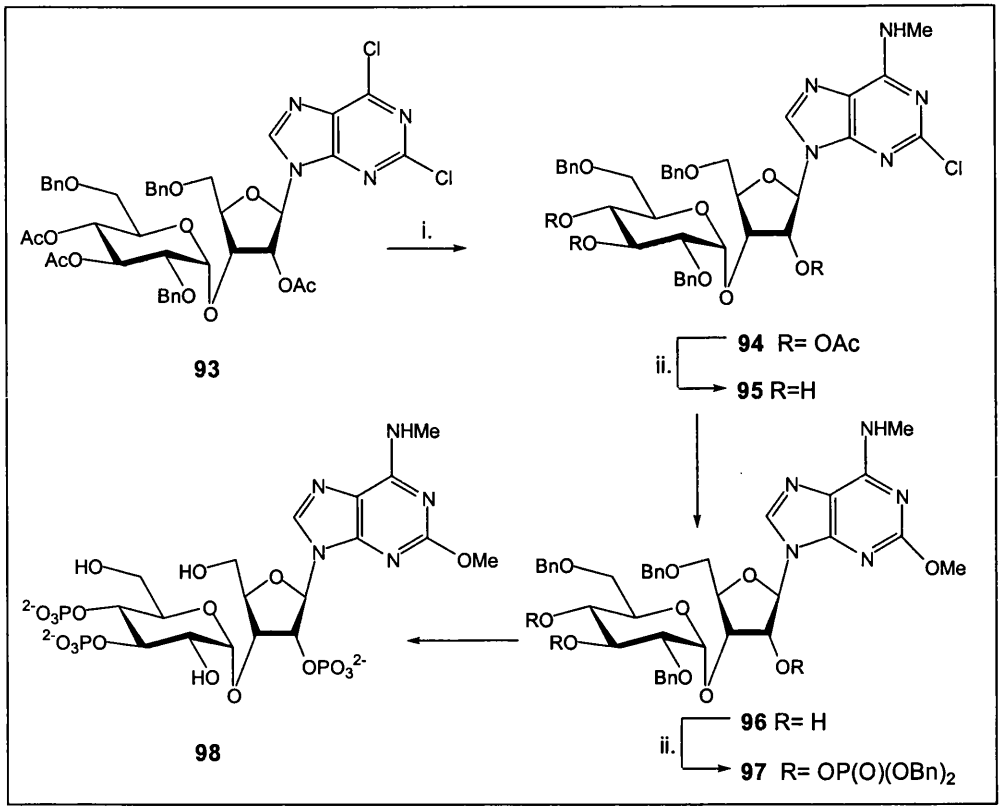


Figure 5.22: Route to 6-NMe, 2-OMe adenophostin.

5.8.3 Phosphorylation and deprotection

The triol (**96**) was phosphitylated with bis(benzyloxy)(diisopropylamino)phosphine and 1*H*-tetrazole, following the usual procedure. Oxidation with *m*CPBA at –78°C gave the trisphosphate triester (**97**). The ^{31}P NMR spectrum of the purified product exhibited three singlets at 0.04, –0.58 and –0.79 ppm corresponding to the three dibenzyloxy phosphate groups.

Deprotection using catalytic transfer hydrogenation as described for the synthesis of adenophostin removed all benzyl protecting groups. The compound was purified by HPLC to give the free acid (**98**), which was quantified by Briggs phosphate assay.

5.9 Synthesis of N6-noradamantylamino adenophostin

The synthesis of noradamantane adenophostin was attempted as the noradamantyl group represents a very large hydrophobe. Initially, the substitution

was attempted on **72** with 3-noradamantanamine hydrochloride, unfortunately the reaction never went to completion and product could not be seen by TLC using variety of eluents. The 6-chlorine atom in **93** seemed more reactive than in **72** so the substitution was attempted. Thus **93** was reacted with noradamantanamine hydrochloride and triethylamine in dichloromethane and ethanol at 60°C overnight. The product was isolated in excellent yield (80%) and identified as a monosubstituted compound.

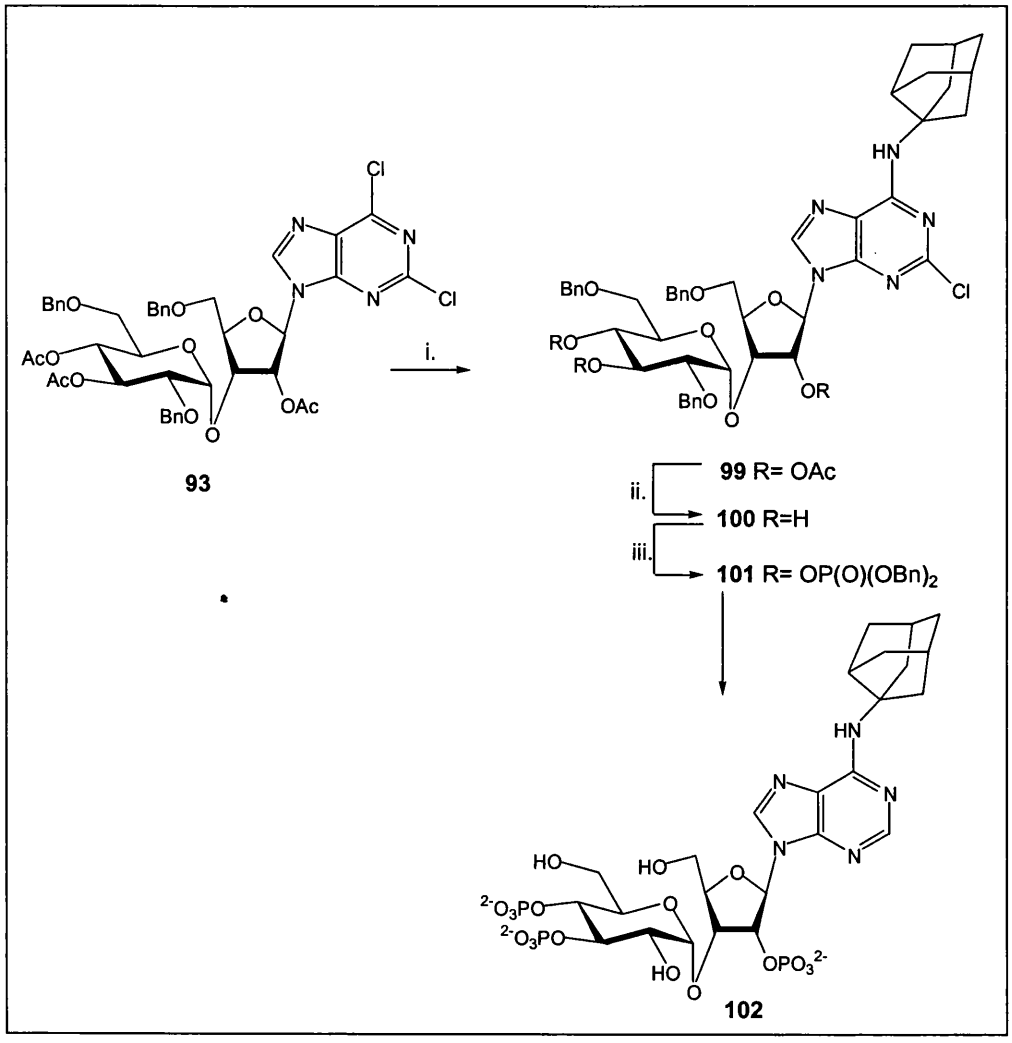


Figure 5.23: Route to noradamantane adenophostin

The acetates of **99** were carefully removed using NaOMe in methanol and followed by TLC to avoid substitution at the 2 position.

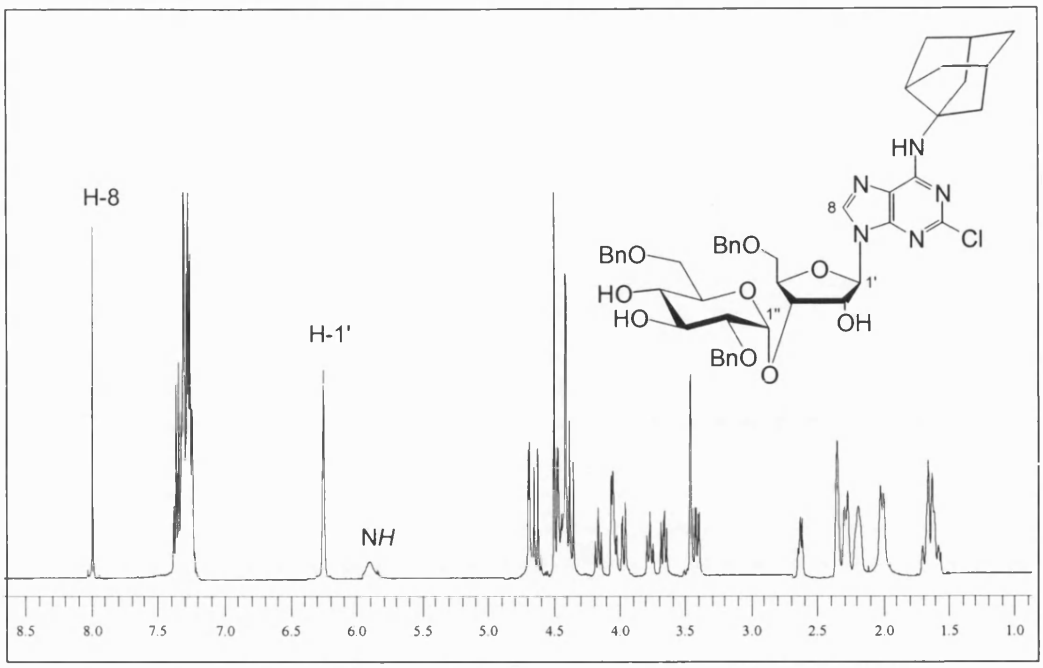


Figure 5.24: part of 400 MHz ^{1}H NMR spectrum of **100** in $CDCl_3$.

5.9.1 Phosphorylation and deprotection

Phosphitylation of **100** with 1*H*-tetrazole and bis(benzyloxy)(diisopropylamino) phosphine in dichloromethane went smoothly to give the trisphosphite which was oxidised to the trisphosphate with *m*CPBA. The ^{31}P NMR spectrum exhibited three characteristic singlets corresponding to the three protected phosphate groups.

Deprotection removed all the benzyl protecting groups and the 2 chlorine atom on the adenine. Thus **101** was heated at reflux in a mixture of water, methanol and cyclohexene with 20% palladium hydroxide on carbon for 2 h. After removal of the catalyst by filtration the crude product was converted to its sodium salt. ^{1}H NMR spectroscopy indicated complete loss of all the benzyl protecting groups and a singlet at 8.03 ppm corresponding to H-2. Quantification was only carried out by the Briggs phosphate assay since this analogue and a sample was examined by HPLC.

5.10 Biological Results

The analogues were biologically tested for Ca^{2+} mobilisation in permeabilised hepatocytes by a collaborator according to the method described in the experimental section. I participated in some of the biological testing. The results are shown in Table **5.2.** Since the analogues were tested in different experiments, the EC_{50} values with respect to the adenophostin ($EC_{50} \cong 10$ nM) values for the individual experiment are given for each analogue. The N6-methyl (**84**), N6-cyclopentyl (**80**) and N6-dimethyl (**88**) analogues were shown to be the most active compounds with an EC_{50} ratio with respect to adenophostin A of approximately 0.80 (<1 is less active than adenophostin A). The N6-noradamantane (**102**) and the methoxy (**76**) analogues also had similar potencies of approximately 0.55 with respect to adenophostin A. The cyclohexyl (**92**) analogue was slighter lower (0.41) and N6-methyl, 2-methoxy (**98**) analogue was the weakest (0.18). Thus, all these compounds are more potent than $Ins(1,4,5)P_3$ and some have activities close to adenophostin A.

Furthermore in the functional studies maximum concentrations of adenophostin A and most of the analogues released the same fraction of the intracellular Ca^{2+} stores (≅34%). Unusually, two compounds (**92** and **102**) released a bigger percentage of the intracellular Ca^{2+} stores (186 ± 21%, 147 ± 9% respectively) in paired controls with Adenophostin A. This is the first time an analogue has released a bigger percentage of the intracellular Ca^{2+} stores, since the cyclopentyl (**80**) analogue did not release the extra amount but the cyclohexyl (**92**) and noradamantane (**102**) analogues did, it seems that as the size of the molecule is increased the ability to release Ca^{2+} is affected.

	EC_{50} nM	*h*	n	% release	EC_{50} wrt **72**
$Ins(1,4,5)P_3$	185 ± 17.90	2.29 ± 0.30	11	35 ± 1.2	0.07
76	18.80 ± 1.90	3.85 ± 1.50	5	34.08 ± 2.64	0.57
80	12.98 ± 1.61	2.15 ± 0.06	5	35.40 ± 1.8	0.81
84	13.24 ± 2.30	2.12 ± 0.44	5	37.03 ± 2.0	0.84
88	19.42 ± 2.09	2.15 ± 0.06	4	38.57 ± 1.96	0.82
92	49.0 ± 5.0	2.33 ± 0.14	6	62.0 ± 4.7	0.27
98	82.06 ± 1.57	8.82 ± 1.57	5	34.21 ± 1.04	0.18
102	34.32 ± 2.04	2.14 ± 0.22	6	49.21 ± 2.10	0.55

Table 5.1: $^{45}Ca^{2+}$ release data for **76**, **80**, **84**, **88**, **92**, **98** and **102** from permeabilised hepatocytes.

The EC_{50} values and Hill coefficients (h) were separately determined for n independent experiments by fitting results to logistic equations. Results are shown as means ± S.E.M.

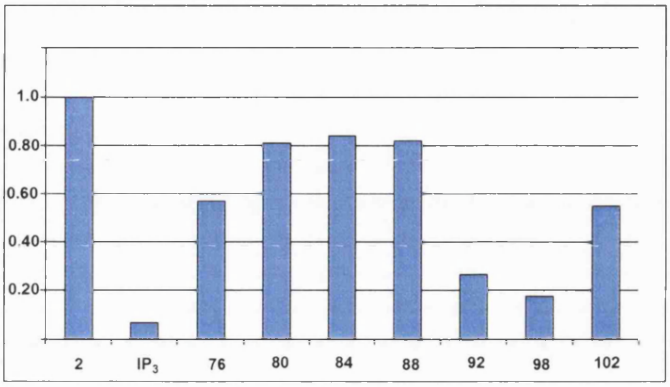
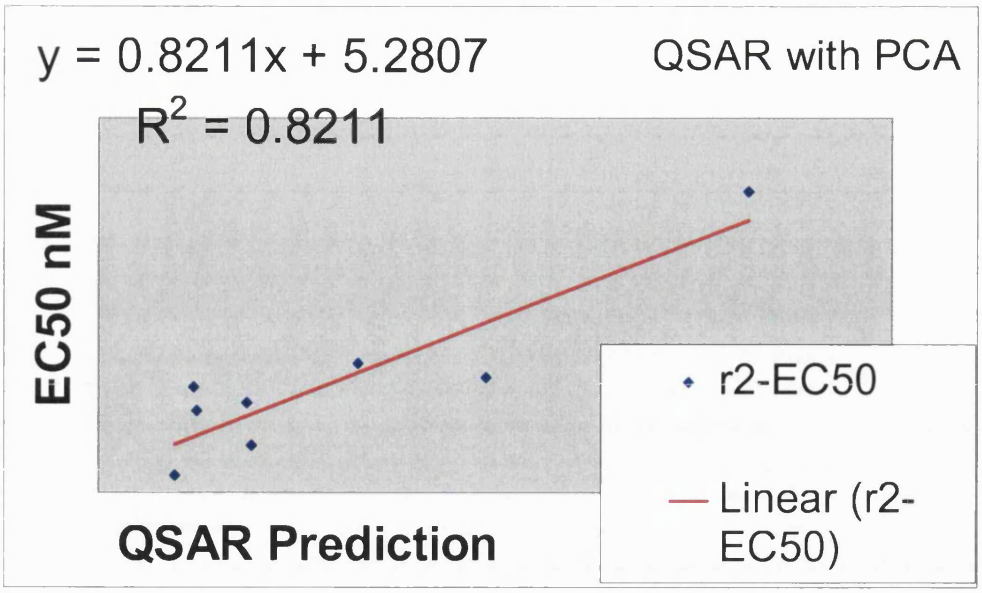


Figure 5.25: Effects of modifying the adenosine moiety of adenophostin A. In this figure the EC50 of each of the analogues is compared with that determined for adenophostin A (**2**) in parallel experiments to give a potency ratio. Ratios <1 denote agonists less potent than adenophostin A.

QSAR studies were attempted on the seven analogues and adenophostin A by Mr. James Robinson. QSAR attempts to find a consistent relationship between the variations in values of molecular properties (descriptors) and the biological activity. A good QSAR should only contain, at most, a third of the number of descriptors to the number of compounds. Energy minima structures were calculated for the eight compounds, using the Merck Forcefield implemented in MOE and descriptors were calculated. Principal component analysis was performed to derive three PCA columns. Partial least squares analysis calculation using the 3 PCA columns resulted in a correlation coefficient of 0.82 and a cross validated coefficient of 0.54. A trend is shown; however, nothing may be quantitatively inferred. With eight compounds and three descriptors the excel spreadsheet data is over fitted. However, it does show there is an underlying relationship between the molecular properties of the compounds and the biological activities. More compounds are needed to establish a firm relationship.



Analogue	EC_{50}	R^2–EC_{50}
Adenophostin	9.60	4.75
98	82.06	80.06
92	49.00	30.75
102	34.32	34.47
76	18.80	23.87
80	12.98	28.04
88	19.42	14.42
84	13.24	21.77

Figure 5.26: QSAR relationship of the seven analogues and adenophostin A.

The energy minima structure was calculated for adenophostin A using MOE, using the Merck Forcefield, MMFF94. Pharmacophore points were chosen based on things like aromatic interactions, hydrogen bond donors and acceptors etc. All the analogues (**76**, **80**, **84**, **88**, **92**, **98**, **102**) were also fitted to the MOE and they are all shown in figure **5.27**.

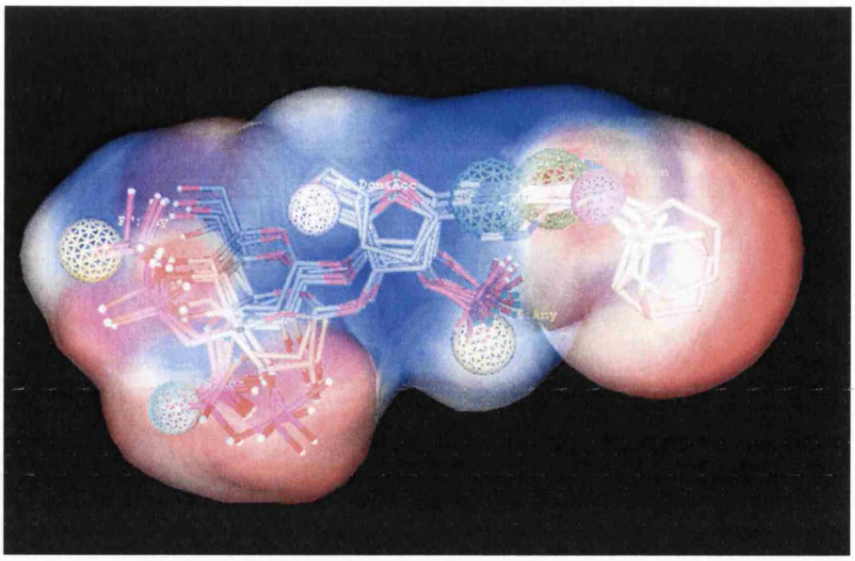


Figure **5.27**: Adenophostin A and analogues (**76**, **80**, **84**, **88**, **92**, **98**, **102**) overlayed in their energy minima structures and surrounded by a charged molecular surface.

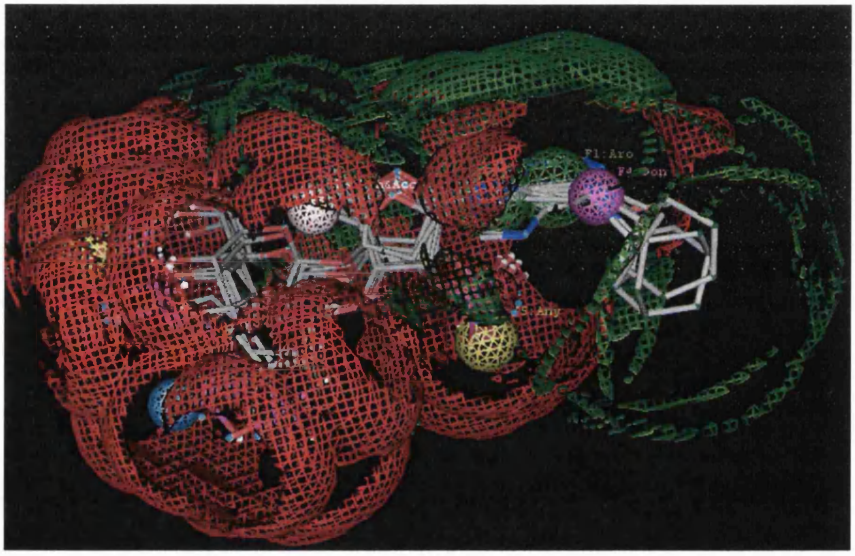


Figure **5.28**: Adenophostin A and analogues (**76**, **80**, **84**, **88**, **92**, **98**, **102**) overlayed in their energy minima structures. The diagram clearly indicates different hydrophilic (red) and hydrophobic (green) regions.

Analysis with methods such as CoMFA, CoMSIA and HQSAR also failed to find suitable QSAR relationships as attaining suitable alignments for the molecules was difficult due to the high degree of flexibility of the molecules analysed.

5.11 Conclusions

These results further demonstrate that a base-modification approach represents a powerful strategy to develop high potency ligands. They also show that elaboration of the adenine ring can indeed produce highly potent compounds. This study, exploring predominantly the introduction of a range of different sized hydrophobes, has revealed evidence of a possible unoccupied and new receptor binding pocket for the Ins(1,4,5)P_3R in complex with adenophostin A. Alternatively the binding site may be exposed at the surface of the receptor allowing the large bulk. However, this seems unlikely as even the large hydrophobic adamantane (**102**) derivative (see modelling fig) has only a slight reduction in potency. The surface of the receptor is likely to be solvated and therefore repel the hydrophobes. Furthermore, by the introduction of a range of different sized hydrophobes we have shown that it is possible to release a greater fraction of the intracellular Ca^{2+} stores. Further work needs to be carried out in order to fully understand these effects.

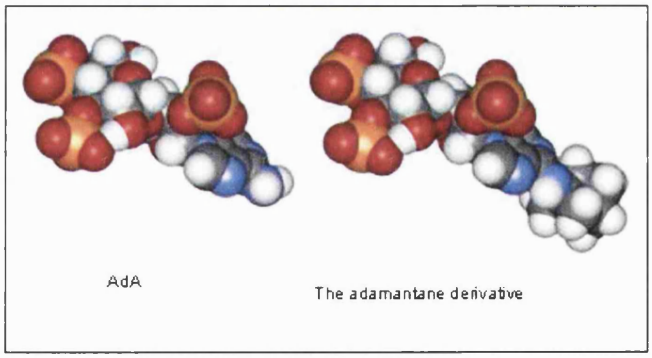


Figure 5.29: molecular modelling

Even though the 2-substituted analogue (**98**) synthesised here has low potency, further 2-modifications need to be carried out to establish whether this site offers similar potential to that at N-6 for reporter group exploitation. Further 2 modifications need to be carried out to determine establish if the decrease in activity is due to conformation, steric or electronic effects.

A useful method for the preparation of a wide variety of 6-substituted and 2,6-disubstituted analogues has been developed. The chemistry needs to be expanded to introduce further hydrophobic groups as well as acidic and basic motifs to determine

weather there is a new binding pocket in the receptor. Some ideas for future work will be discussed in the next chapter.

Chapter Six

Recent developments and general conclusions

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6.1 Recent developments

6.1.1 Biological advances

Since the discovery of the adenophostins biologists have been working hard to determine whether the adenophostins are simply higher affinity analogues of $Ins(1,4,5)P_3$. Adenophostin A is 10-fold more potent at releasing Ca^{2+} than $Ins(1,4,5)P_3$ from internal stores in most functional analyses, whether performed on permeabilised cells, microsomes, planar lipid bilayers, or purified receptors reconstituted into liposomes. Apart from this difference adenophostin A was believed to interact with the $Ins(1,4,5)P_3R$ in a manner identical with that for $Ins(1,4,5)P_3$.

New evidence is currently emerging that suggests that adenophostin A may behave differently from $Ins(1,4,5)P_3$. There are a few subtle differences in the responses evoked by adenophostin A and $Ins(1,4,5)P_3$; Adenophostin A has more positively co-operative interactions in binding assays than $Ins(1,4,5)P_3$; Elementary Ca^{2+} release events, Ca^{2+} puffs, evoked by adenophostin A decay more rapidly than those evoked by $Ins(1,4,5)P_3$. Until recently, these effects were assumed to be a consequence of the ligand dissociating from the $Ins(1,4,5)P_3R$ more slowly than $Ins(1,4,5)P_3$ dissociates. However a recent report showed only a 1.9-fold slower rate of dissociation for adenophostin A than $Ins(1,4,5)P_3$ from the $Ins(1,4,5)P_3R$. The authors speculated that adenophostin A may have an approximately 10-fold faster rate of association with the $Ins(1,4,5)P_3R$ than does $Ins(1,4,5)P_3$[98].

A study very recently published found that under physiological conditions of weak intracellular Ca^{2+} buffering (0.1 mM EGTA), $Ins(1,4,5)P_3$ often fails to activate any detectable store-operated Ca^{2+} current (I_{CRAC}) [99], while adenophostin A consistently activates the current under similar conditions. The analogues *manno*-adenophostin and ribophostin were also tested and it emerged that ribophostin acts in the same way as adenophostin. If the higher affinity of adenophostin A than that of $Ins(1,4,5)P_3$ for the $Ins(1,4,5)P_3R$ is the main factor underlying its ability to activate

I_{CRAC} in weak Ca^{2+} buffer, then neither ribophostin nor *manno*-adenophostin should activate the current in weak buffer, because these analogues have similar affinities to that of Ins(1,4,5)P_3 for the Ins(1,4,5)P_3R. Two possible mechanisms were suggested to account for the preferential activation of I_{CRAC} by adenophostin A and ribophostin in weak buffer in comparison to Ins(1,4,5)P_3. These were; i. different extents of store depletion brought about by different gating properties of the occupied Ins(1,4,5)P_3R or ii. a novel action of adenophostin A and ribophostin in addition to their actions on the Ins(1,4,5)P_3R.

In a recent study it was suggested that ATP affects the activation of the Ins(1,4,5)P_3R by adenophostin A[100]. In the presence of 0.5 mM cytoplasmic free ATP the opening of the Ca^{2+} channel is indistinguishable from Ins(1,4,5)P_3 activation, although the functional affinity of the channel is higher for adenophostin A. However in the absence of ATP both the efficacy and affinity of adenophostin A are significantly reduced. Furthermore, adenophostin A is only a partial agonist under these conditions. The absence of free ATP did not appear to affect the efficacy and affinity of Ins(1,4,5)P_3. The authors suggested that the binding of ATP produces an allosteric conformational change in the ligand-binding site that enhances the binding of adenophostin A to the channel. The 1-phosphoryl group of Ins(1,4,5)P_3 interacts equally well when ATP is present or absent. In the presence of ATP, the 2'-phosphoryl group of adenophostin A can bind to the same phosphoryl group binding site in the receptor that Ins(1,4,5)P_3 interacts with, therefore making adenophostin A indistinguishable from Ins(1,4,5)P_3. However in the absence of ATP the 2'-phosphoryl group of adenophostin A cannot interact in the same way, therefore reducing its efficacy and affinity. The authors also showed that the low affinity adenophostin analogues furanophostin and ribophostin acitvated the Ins(1,4,5)P_3R in a similar way to adenophostin A in either the presence or absence of ATP. It has been proposed that interactions between the adenine of adenophostin A and the Ins(1,4,5)P_3R enhance the affinity of adenophostin A. As adenophostin A and ATP share a common adenine moiety in their molecular structures the authors considered that an interaction of adenophostin A with an ATP binding site in the Ins(1,4,5)P_3R might contribute to high affinity binding. However, their results showed no evidence for ATP being an antagonist competing with adenophostin A for the same binding site in the Ins(1,4,5)P_3R; thus it was concluded that ATP and adenophostin must bind to distinct binding sites in the Ins(1,4,5)P_3R.

$Ins(1,4,5)P_3$ and adenophostin A binding is competitive, and adenophostin A binds with greater affinity than $Ins(1,4,5)P_3$ to each of the $Ins(1,4,5)P_3R$ subtypes. For type 1 and 2 receptors the preference for adenophostin A is greater (7–8 fold) than for type 3 receptor (3 fold). Recently, our biological collaborators [101] have shown that N-terminal fragments of the type 1 receptor bind $Ins(1,4,5)P_3$ with significantly higher affinity than the full length receptor (figure 6.1), but this is not matched by a proportionate increase in their affinity for adenophostin A. In full length receptors, residues lying C-terminal to the $Ins(1,4,5)P_3$-binding core are likely to impede access of the $Ins(1,4,5)P_3$ to its receptor. It was suggested that adenophostin A may be capable of deflecting this inhibitory region to allow less restricted access to the binding pocket and so bind with higher affinity by virtue of more rapid association with the $Ins(1,4,5)P_3R$.

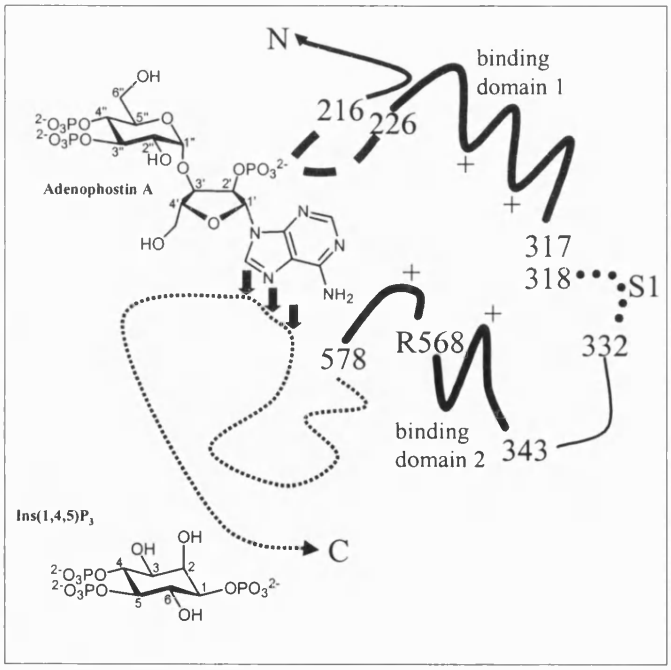


Figure 6.1: Residues 226–576 form the minimal $Ins(1,4,5)P_3$ binding core of the type 1 receptor and within this region several conserved Arg and Lys residues are essential. Both IP_3 and AdA bind to the same binding pocket formed by residues from 2 distinct domains (bold); the two are linked by residues that include the S1 splice site (dotted). Arg-568, in the second of the core domains, is probably essential for recognition of the 1-phosphate of IP_3 and the analogous 2'-phosphate of AdA. Access of both ligands to the binding pocket is partially occluded by residues (dashed) towards the N-terminal. C-terminal residues (thin line) also occlude access to the receptor, but it has been suggested that the adenosine moiety of AdA can bind to this region to displace it and allow fast access of the remainder of the molecule to the core binding domain[101]

At present there are two major explanations to account for the 10-fold greater potency of adenophostin for the Ins(1,4,5)P_3R; i. the adenosine may force the 2'-phosphate into a position that allows it to bind more effectively than the 1-phosphate of Ins(1,4,5)P_3 and/or ii. there may be a direct interaction between the elements of the adenine ring and the Ins(1,4,5)P_3R that contribute to the high affinity.

6.2 Structure-activity relationship work

Along with the synthesis of adenophostin A, the syntheses of thirteen adenophostin A analogues have been presented in this thesis. Their biological evaluation in direct comparison with our synthetic adenophostin A and Ins(1,4,5)P_3 has allowed several important conclusions to be drawn concerning the structure-activity relationships of adenophostin A at the Ins(1,4,5)P_3 receptor. These are summarised in Figure 6.2.

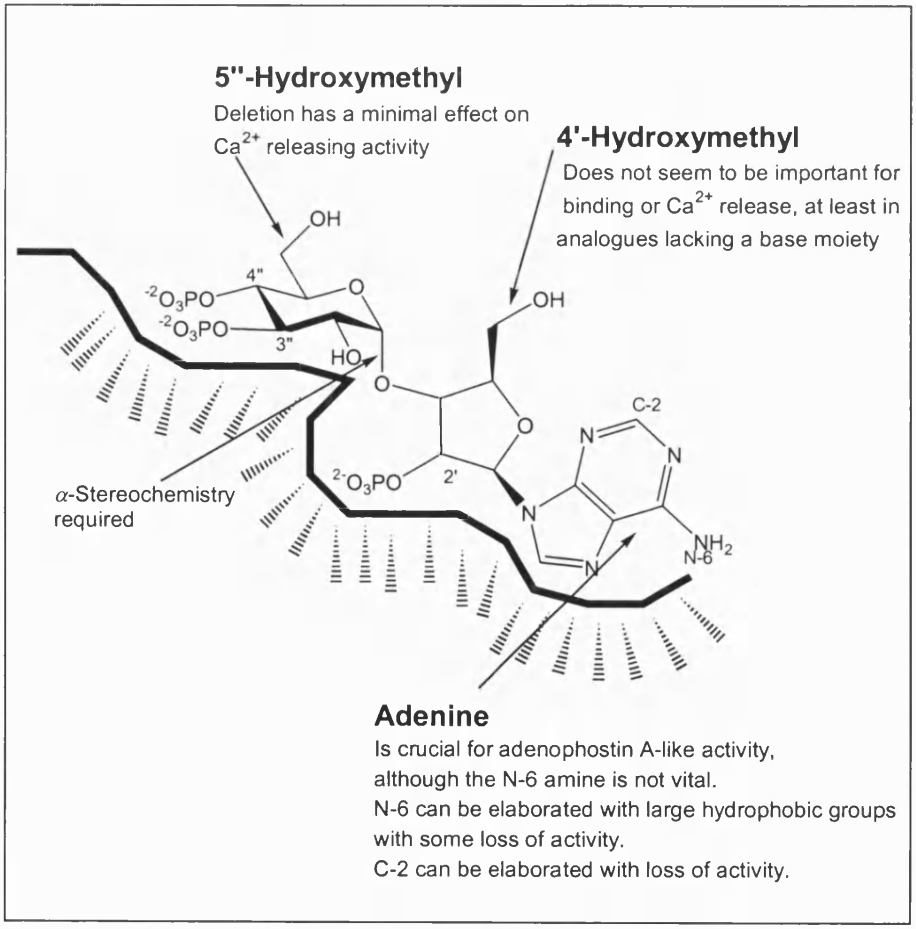


Figure 6.2: The properties of some of the structural features of adenophostin A involved in activity at the Ins(1,4,5)P_3 receptor.

From these results and others it is now well established that the 3",4"-bisphosphate and 2"-hydroxyl group of adenophostin A may effectively mimic the critical 4,5-bisphosphate and 6-hydroxyl groups of Ins(1,4,5)P_3. Combining the results in figure 6.2 and the results from the literature it is possible to further refine these structure activity relationships as shown in figure 6.3.

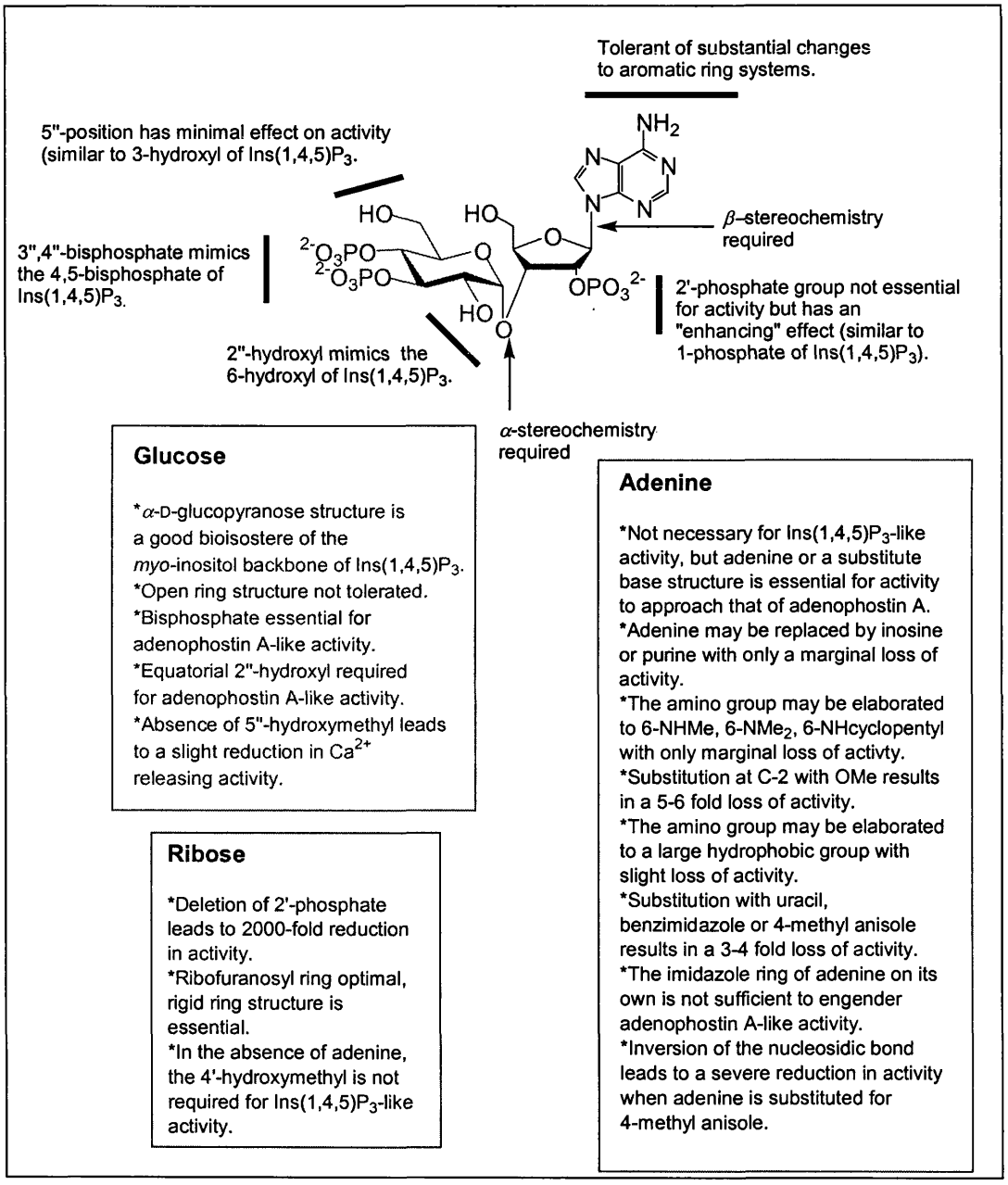


Figure 6.3: Summary of the structure-activity relationships of adenophostin A at the Ins(1,4,5)P_3 receptor.

From the above considerations it is possible to propose a binding model for adenophostin A at the Ins(1,4,5)P_3 receptor. The 3",4"-bisphosphate, adjacent

equatorial 2"-hydroxyl, and well positioned accessory 2-phosphate of adenophostin A comprise the basic structural motif that elicits similar activity to that of Ins(1,4,5)P_3, and thus probably binds in a similar way to the analogous motif of Ins(1,4,5)P_3. For the activity to approach that of adenophostin A an adenine base (base substitute) is vital. The interaction with the adenine base may involve all/some/none of the hydrogen bonds shown in by the dotted lines figure 6.4 alternatively the interaction maybe a hydrophobic one, as even with the introduction of large hydrophobic groups, the potency is still greater than Ins(1,4,5)P_3. There may therefore be an extra binding pocket around the adenine, alternatively it may be fairly open to the solvent. Relating the increased bulk on adenophostin A to Ins(1,4,5)P_3, a number of analogues with a bulky substituent on the C-2 of the inositol ring have been reported[102]. The biological activities of these compounds in a number of systems were only marginally weaker than Ins(1,4,5)P_3. The bulky substitutuent probably occupies the same area in the receptor as the adenine and elaborated adenine of adenophostin A and its analogues.

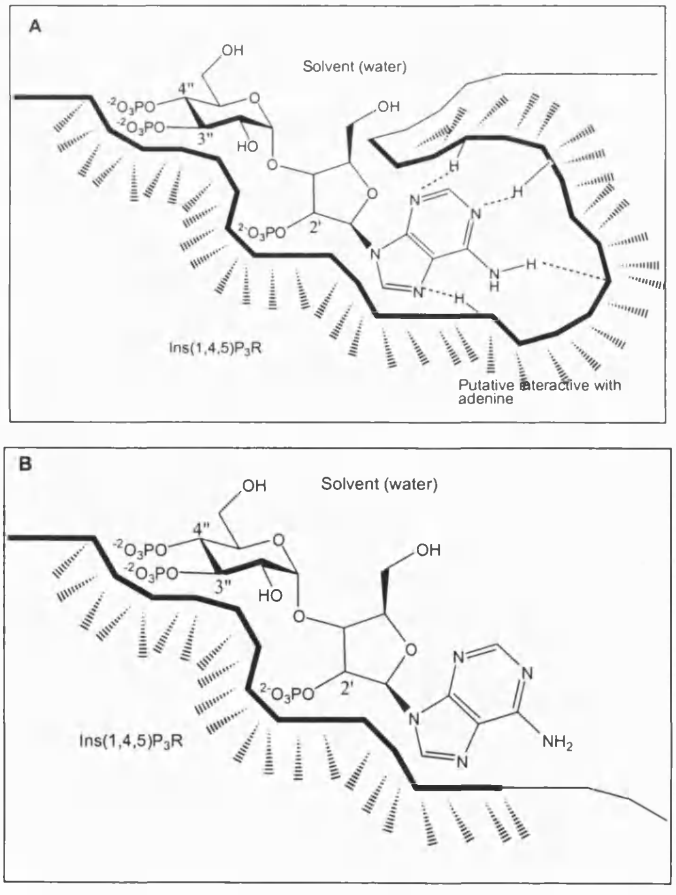


Figure 6.4: Two proposed receptor sites; A. Proposed pocket surrounding the adenine moiety; B. Open to the solvent.

6.3 Future directions

Since the discovery of adenophostin A, syntheses of analogues have given us a better understanding of the structural requirements of adenophostin A that contribute to its exceptional activity; however further research needs to be carried out in order to refine these ideas. The enhanced activity is probably the result of an additional contribution of the adenine.

We have already established the existence of a possible binding pocket around the N-6 position of adenine; this needs further exploration by designing more novel ligands using the 6-chloro intermediate. To obtain more information about the possible existence for a binding pocket further substitutions are required with a range of different properties for example hydrophobic, acidic and basic groups. A strategy was recently published to introduce fluorescent probes into adenosine derivatives. The approach involved an extension of the chemistry described for the synthesis of the 6-substituted compounds as above. Thus 6-chloropurine riboside was reacted with the appropriate mono N-Boc-protected diamine. Subsequent deprotection of the N-Boc group afforded free primary amines which were used in the condensation reaction with the fluorescent tag[103]. Another approach has been to introduce fluorescent labels in the 2-position starting from a guanosine nucleotide [104]. An extension of this strategy could be applied to adenophostin A analogues by altering the protecting group to provide the free primary amine in the final deprotection step, which could then be used to create a wide variety of probes and tags. The probes would be invaluable as a pharmacological tools for studying the complex Ca^{2+} pathways and perhaps novel assets for the location and characterization of Ins(1,4,5)P_3R. Such an approach can also be used to explore further the SAR of adenophostin at the N6 and 2 positions. Extensive generation of modified compounds has previously been severely limited by the long chemistry required for each modification. The whole process could be radically improved if key intermediates modified with pendant amino or sulphurhydryl groups at the N6 and 2-positions could be prepared in large quantity in the fully deprotected forms. It would then be a relatively simple matter using established conjugation chemistry to attach in one step a variety of hydrophobic, acidic and basic motifs to fully explore the potential offered for activity augmentation by either protein site.

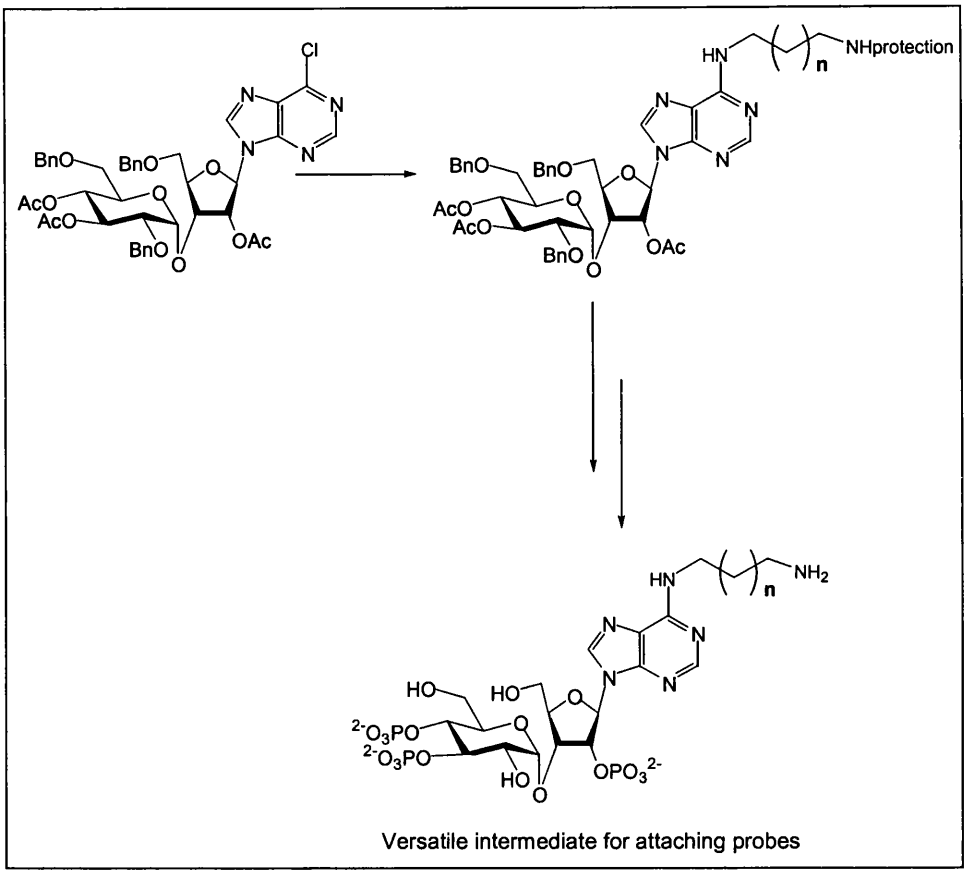


Figure 6.5: Suggested route to versatile intermediate.

Furthermore, an investigation of conformational restriction about the nucleosidic bond would be interesting. The 8-bromo derivative should be accessible directly from adenophostin A and will place the adenine in the syn-configuration, while modifications will enable the synthesis of an anti-configured derivative. Another attractive target, accessible directly from adenophostin A is the fluorescent etheno-derivative (figure 6.6).

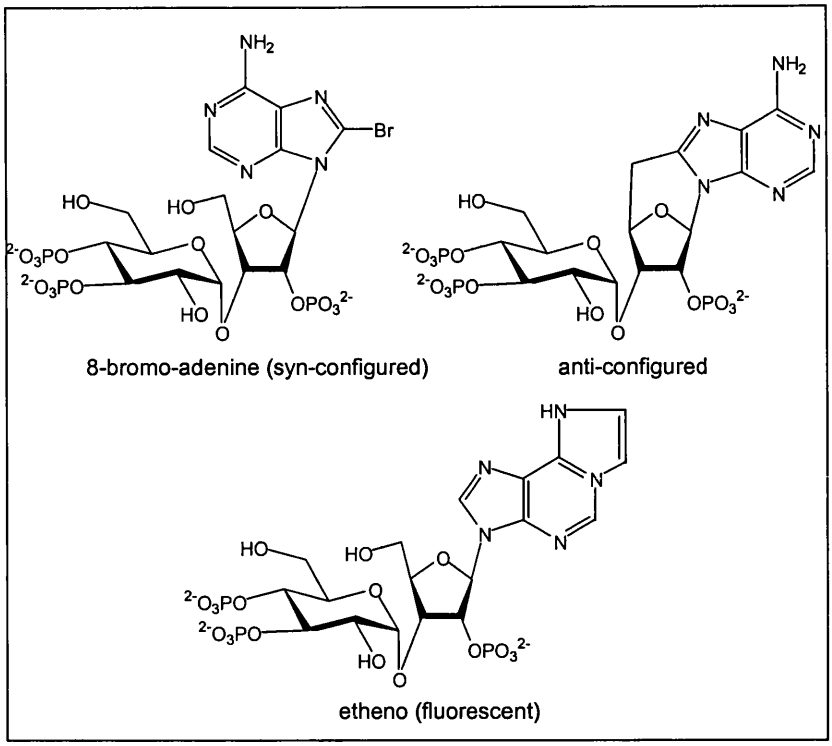


Figure 6.6: Two conformationally restricted analogues and a fluorescent analogue.

Recently, a strategy has been reported for constructing potent ligands for proteins with multiple binding sites[105] The approach involves the synthesis of polymer-linked dimers (PLDs) in which two molecules of the natural ligand are joined by a polymer chain of variable length Applying this approach to tetrameric cyclic nucleotide gated ion channels (CNG channels), it was possible to create PLDs of cGMP whose potency was 1000-fold greater than cGMP itself. This methodology might be applicable to Ins(1,4,5)P_3 receptors which, like CNG channels, are known to be tetrameric ion channels with a separate ligand binding site on each subunit. Recent studies of Ins(1,4,5)P_3 receptors using quick-freeze deep-etch electron microscopy[106;107] have estimated the width of the tetrameric complex to be from 12 and 20 nm; the locations of the Ins(1,4,5)P_3 binding sites are not known. However, by using a convergent synthetic methodology to construct a range of PEG-linked Ins(1,4,5)P_3 dimers it should be possible to cover a range of distances between Ins(1,4,5)P_3 binding sites up to, and possibly exceeding 12 nm. If it is possible to identify a particular size of dimer that gives enhanced affinity for the receptor, not only will this provide a new strategy for developing potent Ins(1,4,5)P_3 receptor ligands, but also structural information on the location of the Ins(1,4,5)P_3 binding

sites in the tetrameric complex. This strategy has recently been employed in the synthesis of Ins(1,4,5)P_3 dimers[108]. Previously, carbohydrate-based Ins(1,4,5)P_3 mimics were attached to a small hydrophobic hub to create bivalent and tetravalent analogues[58;59]. It should be possible to construct more effective dimers by using a more rigid linker (e.g. polypeptide) of the appropriate length, and to extend the methodology to produce dimers of adenophostin A by using the methodology of substituting the 2 or 6 positions of the adenine.

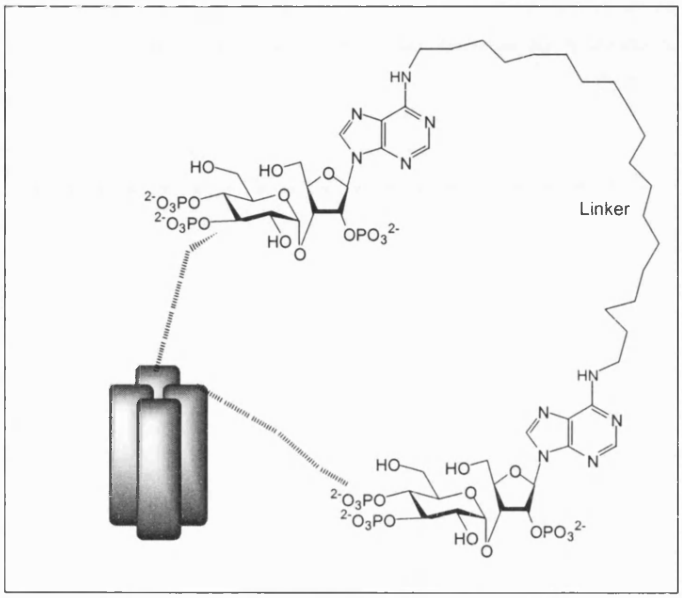


Figure 6.7: Adenophostin A dimers could target multiple receptor binding sites.

Finally it would be useful to synthesise further base substituted compounds; since uridophostin (figure 6.8) was the most potent compound other than those with elaborated adenine bases, it may be useful to synthese the following series based on uridophostin.

With a greater understanding of the properties of the base it should be possible to design analogues of adenophostin A which are more effective modulators of the Ins(1,4,5)P_3 receptor, possibly even antagonists.

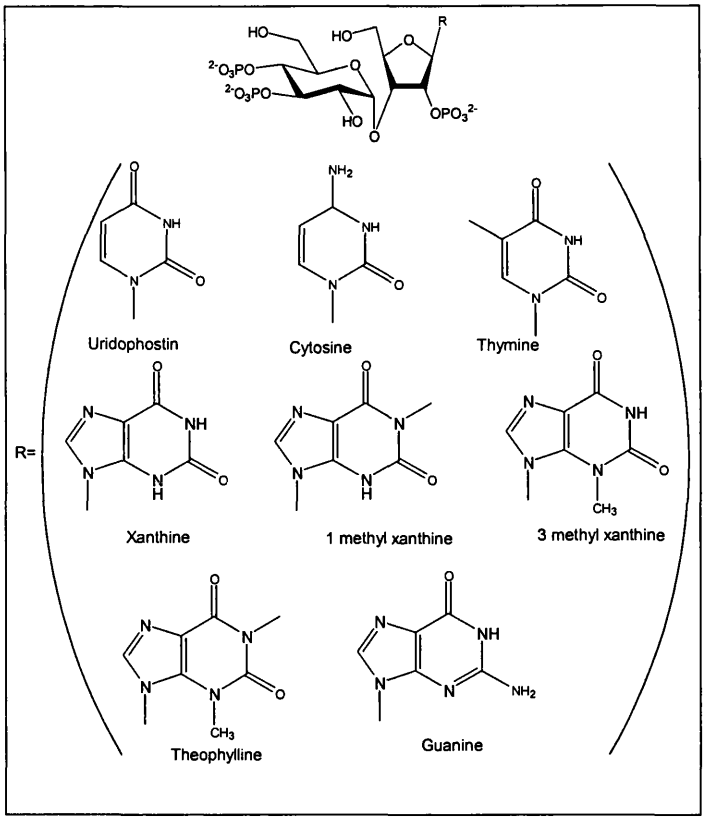


Figure 6.8: Examples of further base substitutions.

Chapter Seven

Experimental

Chapter 7

7 Experimental

7.1 General information

Chemicals were purchased from Acros, Aldrich, Sigma and Fluka. DMF was distilled from barium oxide under reduced pressure and then stored over 4 Å molecular sieves. Pyridine was dried over potassium hydroxide pellets, distilled and then stored over potassium hydroxide pellets. Dichloromethane and acetonitrile were dried over calcium hydride, distilled and then stored over 4 Å molecular sieves. 1,4-Dioxane, dimethyl sulphoxide (DMSO), ethanol, diethyl ether, THF and toluene were purchased in anhydrous form. Ins(1,4,5)P_3 was purchased from American Radiolabeled Chemicals. TLC was performed on precoated plates (Merck aluminum sheets silica 60 F_{254}, Art. No. 5554). Products were visualised under U.V. light and by dipping into phosphomolybdic acid in MeOH followed by heating or by dipping into anisaldehyde in ethanol followed by heating. Flash chromatography was carried out on Silica gel (particle size 40–63 μm). ^{1}H and ^{13}C NMR spectra were recorded on JEOL JMN GX-270 or EX-400 NMR spectrometers. Unless otherwise stated, chemical shifts were measured in ppm relative to internal tetramethylsilane. ^{31}P NMR chemical shifts were measured in ppm and denoted positive downfield from external 85% H_3PO_4.

Melting points were determined using a Reichert-Jung Therm Galen Kofler block and are uncorrected.

Microanalysis was carried out at the University of Bath Microanalysis Service. Low resolution mass spectra were recorded at the University of Bath Mass Spectrometry Service using +ve and –ve fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol as the matrix. High resolution accurate mass spectra were recorded at the University of Bath Mass Spectrometry Service.

Optical rotations were measured at ambient temperature using an Optical Activity Ltd AA-10 polarimeter in a cell volume of 1 mL or 5 mL and specific rotations are given in 10^{-1} deg mL g^{-1}. Ion exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion-Exchange Chromatograph using

Sepharose Q fast flow resin and gradients of triethylammonium bicarbonate as eluent.

HPLC analysis was carried out on a Dynamax model SD-200 with reverse phase column: APEX ODS II 5μ S/N 7121103. A gradient of 0.05M TEAA buffer containing 0.1% tetrabutylammoniumhydrogen sulphate and acetonitrile was used as eluent at 0.9 mL/min, with a UV detector set at 259 nm.

HPLC purification was carried out on a Hewlett Packard series chromatograph with a strong anion exchange resin AGMP1 size 3 mm × 150 mm. A gradient of 150 mM TFA buffer was used as eluent at 1 mL/min, with a UV detector set at 259 nm.

Synthetic phosphates were assayed by adaptations of the Briggs phosphate[80] test as follows. For the qualitative test, 200 μL aliquots of the ion exchange column fractions were pipetted into a test tube and 4 drops of conc. sulfuric acid added to each tube, which was then heated to an oven at 200°C for 1 hour. The tubes were allowed to cool and water (200 μL) was added to dissolve the residue. An aliquot (400 μL) of a solution of ammonium molybdate (5 g) in water (40 ml) and conc. sulphuric acid (16 ml) was added, followed by an aliquot (200 μL) of a solution of quinol (200 mg) in water (40 ml) and two drops of conc. sulphuric acid, then finally an aliquot (200 μL) of a solution of sodium sulphite (8 g) in water (40 ml) was added. The mixture was heated to boiling for 10 seconds. A blue colour indicated the presence of inorganic phosphate.

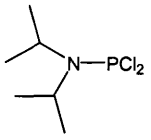
For the quantitative assay the mixtures treated as above were transferred to volumetric flasks and made up to 10 mL with water. The UV absorbances at 340nm were recorded using 3 mL quartz cells. Concentration was calculated from a standard curve compiled from UV absorbance values of known concentrations of KH_2PO_4 treated as above and measured at 340nm.

The method used by our collaborators for the biological testing was as follows: Hepatocytes were isolated by collagenase digestion of the livers of male Wistar rats and stored at 4 °C in Eagles medium supplemented with 26 mM $NaHCO_3$ and BSA (2% w/v) for up to 24 h. The Eagles medium was removed and the cells were resuspended in a cytosol-like medium (CLM: KCl, 140 mM, NaCl, 20 mM, 2 mM $MgCl_2$, 1mM EGTA, 300μM $CaCl_2$, 20mM Pipes, pH7.0) where they were permeabilised by incubation with saponin (10 μg/mL). They were subsequently loaded to steady state (5 min at 37°C) with $^{45}Ca^{2+}$ in a cytosol-like medium containing ATP (1.5 mM), creatine phosphate (5 mM) creatine phosphokinase (5 units/mL) and FCCP (10 μM). After 5 min, thapsigargin (1.25 μM) was added to the cells to inhibit further Ca^{2+} uptake (by inhibiting the activity of the SERCA pump), 30s later the cells were added to appropriate concentrations of the

agonists and after a further 60s the $^{45}Ca^{2+}$ contents of the stores were determined by rapid filtration. Concentration-response relationships were fitted to a four parameter logistic equation using Kaleidegraph software (Synergy Software, PA) from which the maximal response, half-maximally effective agonist concentration (EC_{50}) and Hill slope (h) were determined. All results are expressed as means ± SEM.

7.2 Synthesis of Bis(benzyloxy)diisopropylaminophosphine

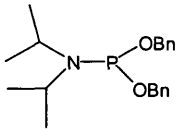
N,N-Diisopropylaminodichlorophosphine (19).



Dry *N,N*-diisopropylamine (141 mL, 1.4 mol) in dry diethyl ether (150 mL) was added dropwise to a stirred solution of phosphorus trichloride (44 mL, 0.32 mol) in dry diethyl ether (100 mL) at –78 °C. More dry diethyl ether (300 mL) was added and the mixture was stirred overnight at room temperature. The precipitated amine salt was then filtered and washed with 300 mL of diethyl ether and concentrated to give an oil. The crude mixture was distilled, b.p. 62–63 °C at 7 mmHg (lit b.p. 62–63 °C[79]) to give the title compound (57 g, 89%). The product was stored at –20 °C.

^{1}H NMR (270 MHz; $CDCl_3$) δ_H 3.97 (septet, 2 H, J 6.5 Hz, 2 × $(CH_3)_2CHN$) 1.33 (d, 12 H, J 6.9 Hz, 2 × $(CH_3)_2C(H)N\text{-}PCl_2$); ^{31}P NMR (1.09 MHz, $CDCl_3$), δ_P 172.88:

Bis(benzyloxy)diisopropylaminophosphine (20).

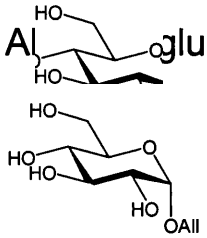


A mixture of **19** (14.44g, 0.072 mmol) in dry dichloromethane (160 mL) and dry triethylamine (21.1 mL, 0.151 mmol) was stirred at –78 °C. Anhydrous benzyl alcohol (14.8 mL, 0.14 mmol) was added dropwise and stirred for a further 2h. The amine salt was filtered and washed with a further two portions of dichloromethane (40 mL). The organic portion was then washed with satd aq. $NaHCO_3$ (150 mL) and H_2O (150 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave a yellow oil, which was purified by flash chromatography on silica using hexane–triethylamine (10:1) as eluent to give the title compound (20.1 g, 81%) as a colourless oil.

^{1}H NMR (270 MHz; $CDCl_3$) δ_H 7.47–7.33 (m, 10 H, Ar*H*), 4.92–4.78 (m, 4 H, 2 × CH_2Ar), 3.85–3.76 (m, 2 H, 2 × $(CH_3)_2CHNPOCH_2Ar$ and 1.20 (d, 12 H, J 7.0 Hz, 2 × $(CH_3)_2CHNPOCH_2Ar$); ^{31}P NMR (1.09 MHz, $CDCl_3$), δ_P 149.03:

7.3 Synthesis of the *C*-Glycoside trisphosphates

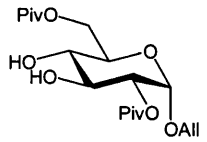
Allyl α-D-glucopyranoside (3).



Acetyl chloride (4 mL, 70 mmol) was added dropwise to allyl alcohol (500 mL, 7.33 mol) and the mixture was stirred at RT for 15 min. D-glucose (100 g, 0.56 mol) was added and the mixture was refluxed heated at for 3 h. The mixture was cooled, neutralised with $NaHCO_3$ (10 g), filtered and concentrated under reduced pressure to give a viscous orange syrup. It was purified by flash chromatography on silica using ethyl acetate–methanol (9:1) as eluent to give a white compound. This was crystallised from hot acetone (500 mL) to give the title compound (37.3 g, 30.5%).

m.p. 94-96 °C; Lit m.p. 99-100 °C[46]; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 6.01–5.89 (m, 1 H, CH_2CH=CH_2), 5.33 (d, 1 H, ^3J 17.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.25 (d, 1 H, ^3J 10.4 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.94 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.25–4.02 (m, 2 H, CH_2CH=CH_2), 3.85–3.65 (m, 4 H, H-3, H-5, H-6a, H-6b), 3.53 (dd, 1 H, $J_{2,1}$ 3.8 Hz, $J_{2,3}$ 9.9 Hz, H-2) and 3.38 (t, 1 H, $J_{4,3}$=$J_{4,5}$ 9.7 Hz, H-4):

Allyl 2,6-di-O-trimethylacetyl-α-D-glucopyranoside (4).

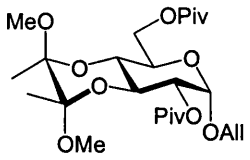


A solution of **3** (5.0 g, 22 mmol) was dissolved in dry pyridine (50 mL) under N_2. The solution was cooled to 0 °C and trimethylacetyl chloride (5.6 mL, 45 mmol) was added dropwise over 1 h. Stirring was continued for a further 1 h. The mixture was poured into ice water (200 mL) and extracted with diethyl ether (200 mL). The organic solution was washed successively with 1 M HCL (200 mL) and sat. aq. $NaHCO_3$ solution (200 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give crude **4**, which was used in the next step without further purification. A small sample was subjected to flash chromatography on silica using diethyl ether–hexane (3:1) as eluent to give the title compound as an oil.

$[\alpha]^{20}_D$ +90.6 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.89–5.79 (m, 1 H, CH_2CH=CH_2), 5.28 (dd, 1 H, ^2J 1.5 Hz, ^3J 17.1 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.17 (dd, 1 H, ^2J 0.9 Hz, ^3J 10.3 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.02 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.61 (dd, 1 H, $J_{2,1}$ 3.9 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.42 (dd, 1 H, $J_{6a,5}$ 4.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.33 (dd, 1 H, $J_{6b,5}$ 1.9 Hz, $J_{6b,6a}$ 12.2 Hz, H-6b), 4.19–4.14 (m, 1 H, CH_2CH=CH_2), 4.09–3.92 (m, 3 H, H-3, CH_2CH=CH_2), 3.85–3.80 (m, 1 H, H-5), 3.38 (t, 1 H, $J_{4,3}$=$J_{4,5}$ 9.3 Hz, H-4) and

1.22 (s, 18 H, 2 × C(O)C(CH_3)$_3$); ^{13}C NMR (67 MHz; $CDCl_3$) δ_C 179.40, 178.45 (2 × C(O)C(CH_3)$_3$), 133.42 (CH_2CH=CH_2), 117.59 (CH_2CH=CH_2), 95.09 (C-1), 72.76, 71.50, 70.76, 69.09,(4 × CH), 68.40 (CH_2CH=CH_2), 63.22 (C-6), 38.97 (C(O)C(CH_3)$_3$), 27.21 (C(O)C(CH_3)$_3$) and 27.07 (C(O)C(CH_3)$_3$); MS: (FAB) *m/z* 799.6 [(2M+Na)$^+$, 85%], 777.6 [(2M+H)$^+$, 59%], 411.3 [(M+Na)$^+$, 20%]; Anal. Calcd for $C_{19}H_{32}O_8$: C, 58.75; H, 8.30%. Found: C, 58.80%; H 8.32%.

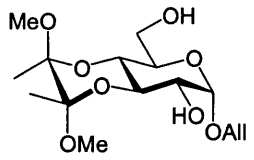
(2'S,3'S) allyl 3,4-di-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-2,6-di-*O*-trimethylacetyl-α-*D*-glucopyranoside (5).



A solution of **4** (1.78 g, 4.58 mmol), trimethyl orthoformate (4.2 ml, 38 mmol) butane-2,3-dione (1.43 mL, 16.3 mmol) and CSA (20 mg, 0.086 mmol) in methanol (10 mL) was heated under reflux for 3 h, when TLC (diethyl ether) showed consumption of starting material and showed the presence of one product. The mixture was allowed to cool to RT and triethylamine was added until the mixture was neutralised. The mixture was concentrated under reduced pressure and the syrup thus obtained was subjected to flash chromatography on silica using dichloromethane–acetone (20:1) as eluent to give the title compound (1.78 g, 62.5 %) as an oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.90–5.76 (m, 1 H, CH_2CH=CH_2), 5.23 (dd, 1 H, ^2J 1.6 Hz, ^3J 17.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.15 (dd, 1 H, ^2J 1.6 Hz, ^3J 10.4 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.10 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.73 (dd, 1 H, $J_{2,1}$ 3.9 Hz, $J_{2,3}$ 10.6 Hz, H-2), 4.45 (dd, 1 H, $J_{6a,5}$ 2.2 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.27–4.19 (m, 1 H, H-3 or H-4), 4.17–3.89 (m, 4 H, H-5, H-6b, CH_2CH=CH_2), 3.73–3.60 (m, 1 H, H-3 or H-4), 3.31 (s, 3 H, OCH_3), 3.24 (s, 3 H, OCH_3), 1.30 (s, 3 H, CH_3), 1.21 (s, 3 H, CH_3) and 1.20 (s, 18 H, 2 × C(O)C(CH_3)$_3$); ^{13}C NMR (67 MHz; $CDCl_3$) δ_C 178.14, 178.24 (2 × C(O)C(CH_3)$_3$), 133.59 (CH_2CH=CH_2), 117.24 (CH_2CH=CH_2), 99.72, 99.65 (BDA OCCH_3OCH_3) 95.18 (C-1), 72.779 (CH), 70.48 (CH_2CH=CH_2), 68.30, 67.70, 66.90,(3 × CH), 62.11 (C-6), 48.09, 47.73 (2 × OCH_3), 38.77 (C(O)C(CH_3)$_3$), 27.06 (C(O)C(CH_3)$_3$), 27.23 (C(O)C(CH_3)$_3$) and 17.73, 17.58 (2 × CH_3); MS: (FAB) *m/z* 525.4 [(M+Na)$^+$, 68%]:

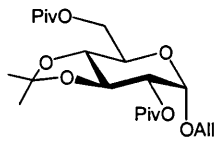
(2'S,3'S) Allyl 3,4-di-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-*α*-*D*-glucopyranoside (6).



A solution of crude **5** (2.85 g, 5.76 mmol), NaOH (0.93 g, 23 mmol) in methanol (100 mL) was heated under reflux for 1 h. The mixture was cooled, neutalised with solid CO_2. The solvents were evaporated off under reduced pressure and the residue was partitioned between diethyl ether (100 mL) and water (60 mL). The aqueous layer was back-extracted with diethyl ether (100 mL) and the combined organic fractions were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil which was subjected to flash chromatography on silica using diethyl ether as eluent. (545 mg, 28% over 2 reactions).

$[\alpha]^{20}_D$= +299 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.92–5.84 (m, 1 H, CH_2CH=CH_2), 5.29 (dd, 1 H, ^2J 1.6 Hz, ^3J 17.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.22 (dd, 1 H, ^2J 1.3 Hz, ^3J 10.3 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.96 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.25–4.10 (m, 2 H, CH_2CH=CH_2), 3.98–3.90 (m, 1 H, H-3 or H-4), 3.84–3.71 (m, 5 H, H-2, H-3 or H-4, H-5, H-6a, H-6b), 3.31 (s, 3 H, OCH_3), 3.26 (s, 3 H, OCH_3), 1.34 (s, 3 H, CH_3) and 1.30 (s, 3 H, CH_3); ^{13}C NMR (67 MHz; $CDCl_3$) δ_C 133.00 (CH_2CH=CH_2), 118.00 (CH_2CH=CH_2), 99.62, 99.61 (BDA $OCCH_3OCH_3$) 97.67 (C-1), 70.29, 69.87 (2 × CH), 68.74 (CH_2CH=CH_2), 65.96, (CH), 61.55 (C-6), 48.02, 47.97 (2 × OCH_3), and 17.76, 17.66 (2 × CH_3); MS: (FAB) *m/z* 357.3 [$(M+Na)^+$, 15%]; Anal. Calcd for $C_{15}H_{26}O_8$ C, 53.88; H, 7.84%. Found: C, 54.0; H, 7.95%:

Allyl 2,6-di-*O*-trimethylacetyl-3,4-*O*-isopropylidene-*α*-*D*-glucopyranoside (7).

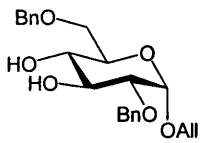


A solution of crude **4** (8.82 g, 22.0 mmol), 2-methoxypropene (5.5 mL, 56 mmol) and *p*-toluenesulphonic acid (100 mg) in THF (100 mL) was stirred for 2 h at RT under N_2. TLC diethyl ether–hexane (1:1) indicated consumption of starting material. Diethyl ether (250 mL) was added and the mixture was washed with sat. aq. $NaHCO_3$ solution (250 mL). The organic layer was dried ($MgSO_4$) and filtered, and a few drops of Et_3N were added before the organic layer was concentrated under reduced pressure. The product of this reaction was used directly in the next step without purification. A small sample was purified by flash

chromatography on silica using diethyl ether–hexane (1:2) as eluent to give the title compound for analysis.

$[\alpha]^{20}_D$ +114 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$) δ_H 5.92–5.78 (m, 1 H, CH_2CH=CH_2), 5.28 (dd, 1 H, ^2J 1.7 Hz, ^3J 17.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.20–5.17 (m, 2 H, H-1, CH_2CH=$CH_{cis}H_{trans}$), 4.83 (dd, 1 H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.6 Hz, H-2), 4.37 (dd, 1 H, $J_{6a,5}$ 2.2 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.20–4.10 (m, 1 H, H-6b), 4.18–3.91 (m, 4 H, H-3, H-5, CH_2CH=CH_2), 3.37–3.30 (m, 1 H, H-4), 1.44 (s, 3 H, isopropylidene CH_3), 1.42 (s, 3 H, isopropylidene CH_3), 1.23 (s, 9 H, (C(O)C(CH_3)$_3$) and 1.22 (s, 9 H, (C(O)C(CH_3)$_3$); ^{13}C NMR (67 MHz; $CDCl_3$) δ_C 177.98 (2 × *C*(O)C(CH_3)$_3$), 133.39 (CH_2CH=CH_2), 117.80 (CH_2CH=CH_2), 111.00 (isopropylidene *C*(CH_3)$_2$), 95.32 (C-1), 72.98, 71.50, 70.68, 69.76 (4 × CH), 69.84 (*C*H_2CH=CH_2), 63.50 (C-6), 27.00, 27.16, 26.40 and 26.70 (2 × isopropylidene *C*H_3, 2 × (C(O)C(*C*H_3)$_3$); MS: (FAB) *m/z* 429.0 [$(M+H)^+$, 97%]; Anal. Calcd for $C_{22}H_{36}O_8$ C, 61.66%; H, 8.47%. Found: C, 61.90%; H, 8.35%.

Allyl 2,6-di-*O*-benzyl-α-*D*-glucopyranoside (10).

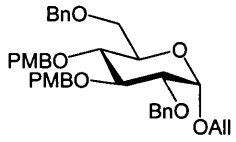


A solution of crude **7** (9.77 g, 22 mmol), NaOH (3.6 g, 90 mmol) in methanol (50 mL) was heated under reflux for 1 h. The mixture was cooled to RT and the pH was adjusted to 8 by careful addition of solid CO_2. Following concentration *in vacuo* the residue was partitioned between diethyl ether (100 mL) and water (50 mL). The aqueous layer was back-extracted with diethyl ether (10 mL) and the combined organic fractions were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The oil obtained was dissolved in dry DMF (40 mL) and was stirred at 0 °C with NaH (2.25 g of a 60 % w/w dispersion in mineral oil, 56 mmol) and benzyl bromide (6.1 mL, 56 mmol) was added slowly. The mixture was stirred at RT for 90 min. Water (50 mL) was added and stirring continued for 60 min. The solvents were evaporated under reduced pressure and the residue was dissolved in diethyl ether (200 mL). The extract was washed with water (500 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The oil was dissolved in methanol (50 mL) and stirred with 1 M HCl (5 mL) for 30 min. TLC diethyl ether–hexane (1:2) indicated consumption of starting material. Solid $NaHCO_3$ was added until the mixture reached neutral pH. The solvents were evaporated off and the residue was partitioned between dichloromethane (100 mL) and water (50 mL). The aq. layer was back extracted with CH_2Cl_2 (200 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The product was

crystallised from diethyl ether–hexane to give the title compound (6.1 g, 67% over the previous 5 steps from **3**).

$[\alpha]^{20}_D$ +76.3 (*c* 0.8, $CHCl_3$); Lit $[\alpha]^{20}_D$ +76.4 (*c* 0.8, $CHCl_3$)[46]; m.p. 75-77 °C; Lit m.p. 74-77 °C[46]; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 7.36–7.26 (m, 10 H, ArC*H*), 5.94–5.83 (m, 1 H, CH_2CH=CH_2), 5.31 (dd, 1 H, ^2J=1.6, ^3J=17.2, CH_2CH=$CH_{cis}H_{trans}$), 5.23 (dd, 1 H, ^2J 1.6 Hz, ^3J 10.3 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.84 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.67, 4.62 (AB, 2 H, J_{AB} 12.1 Hz, ArCH_2O), 4.59, 4.54 (AB, 2 H, J_{AB} 12.1 Hz, ArCH_2O), 4.18–4.11 (m, 1 H, CH*H*CH=CH_2), 3.98–3.90 (m, 2 H, H-3 C*H*HCH=CH_2), 3.78–3.68 (m, 3 H, H-5, H-6a, H-6b), 3.62 (t, 1 H, $J_{4,3}$=$J_{4,5}$ 9.2 Hz, H-4) and 3.38 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 9.5 Hz, H-2):

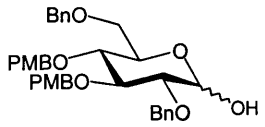
Allyl 2,6-Di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-*D*-glucopyranoside (11).



A solution of **10** (6 g, 15 mmol) in dry DMF (40 mL) was stirred with sodium hydride (2.39 g, 60 mmol) and *p*-methoxybenzyl chloride (8.1 mL, 60 mmol) at RT for 3 h. Methanol (30 mL) was added and stirring continued for 15 min. The solvents were concentrated under reduced pressure and the residue was dissolved in diethyl ether. The organic solution was washed with water, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica using hexane–ethyl acetate (19:1) as eluent to give the title compound as an oil.

^{1}H NMR (270 MHz; $CDCl_3$) δ_H 7.34–7.25 (m, 12 H, ArC*H*, H-2 of PMB ring, H-6 of PMB ring), 7.04 (d, 2 H, *J* 8.6 Hz, H-2 and H-6 of PMB ring), 6.90–6.78 (m, 4 H, 2 × H-3 of PMB rings, 2 × H-5 of PMB rings), 5.93–5.91 (m, 1 H, CH_2CH=CH_2), 5.29 (d, 1 H, ^3J=17.2, CH_2CH=$CH_{cis}H_{trans}$), 5.19 (d, 1 H, ^3J=10.3, CH_2CH=$CH_{cis}H_{trans}$), 4.92–4.36 (m, 9 H, 4 × ArCH_2O, H-1), 4.12–3.97 (m, 3 H, H-3, CH_2CH=CH_2), 3.80 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3) and 3.72–3.51 (m, 5 H, H-2, H-4, H-5, H-6a and H-6b):

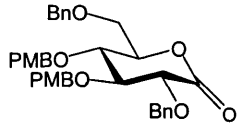
2,6-Di-O-benzyl-3,4-di-O-(p-methoxybenzyl)-α-d-glucopyranoside (12).



11 (5.5 g, 8.6 mmol) In methanol (50 mL) was stirred vigorously with $PdCl_2$ (304 mg, 1.7 mmol) for 3 h. TLC dichloromethane–acetone (10:1) showed consumption of the starting material. Et_3N (1 mL) was added to neutralise the reaction. It was filtered through Celite, the solvents were evaporated off and the residue was subjected to flash chromatography on silica using dichloromethane–acetone (10:1) as eluent to give a pale yellow oil which was crystallised from diethyl ether to give the title compound as a white solid (3.59 g, 70.5 %).

^{1}H NMR (270 MHz; $CDCl_3$) δ_H 7.35–7.25 (m, 12 H, ArC*H*, H-2 of PMB ring, H-6 PMB ring), 7.07–7.04 (m, 2 H, H-2 of PMB ring, H-6 of PMB ring), 6.87–6.78 (m, 4 H, 2 × H-3 of PMB rings, 2 × H-5 of PMB rings), 5.21 (t, 0.5 H, *J* 2.6 Hz, H-1$_\alpha$), 4.88–4.39 (m, 8.5 H, H-1$_\beta$, 4 × ArCH_2O), 3.92 (t, 0.5 H, $J_{3,2}$=$J_{3,4}$ 9.3 Hz, H-3$_\alpha$ or H-3$_\beta$), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.67–3.53 (m, 5.5 H, 0.5 × H-2$_\alpha$, H-3$_\alpha$ or H-3$_\beta$, H-4, H-5, H-6a, H-6b) and 3.44–3.30 (m, 0.5 H, H-2$_\beta$):

2,6-Di-O-benzyl-3,4-di-O-(*p*-methoxybenzyl)-α-D-glucono-1,5-lactone (13).

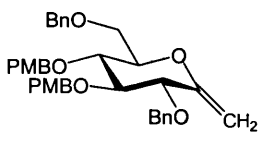


Dry dichloromethane (1 mL) was placed into a 100 mL flask under at atmosphere of N_2. A solution of oxalyl chloride in dry dichloromethane (1.7 mL, 3.4 mmol of 2 M solution) was injected and the flask was cooled to –78 °C. Anhydrous dimethyl sulphoxide (0.5 mL, 7 mmol) dissolved in dry dichloromethane was added dropwise (care evolution of gas) and stirring was continued for 5 min. A solution of **12** (2.0 g, 3.3 mmol in 2 mL dry dichloromethane) was added dropwise and stirring was continued for 20 min. Et_3N (1.8 mL, 13 mmol) was added dropwise and the reaction was stirred for a further 5 min, then the mixture was allowed to heat up to RT. The mixture was stirred with water (40 mL) for 10 min, then dichloromethane (80 mL) was added. The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (80 mL). The combined organic layers were washed successively with sat. aq. NaCl (80 mL), 1% HCl (80 mL) and 10% aq. $NaHCO_3$ (80 mL), dried and concentrated under reduced pressure.

The residue was subjected to flash chromatography on silica using diethyl ether–hexane (1:1) as eluent to give the title compound as an oil (1.33 g, 67%).

$[\alpha]^{20}_D$ +64.7 (*c* 0.5, $CHCl_3$); IR; υ 1755cm^{-1} (carbonyl); ^{1}H NMR (400 MHz; $CDCl_3$;) δ_H 7.39–7.07 (m, 14 H, ArC*H*, 2 × H-2 of PMB ring, 2 × H-6 of PMB ring), 6.86–6.80 (m, 4 H, 2 × H-3 of PMB ring, 2 × H-5 of PMB ring), 4.98 (AB, 1 H, J_{AB} 11.42 Hz, 0.5 × ArCH_2O), 4.67–4.40 (m, 8 H, H-5, 3.5 × ArCH_2O), 4.09 (d, 1 H, $J_{2,3}$ 6.2 Hz, H-2), 3.95–3.85 (m, 2 H, H-3, H-4), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.69 (dd, 1 H, $J_{6b,6a}$ 10.8 Hz, $J_{6b,5}$ 2.3 Hz, H-6b) and 3.63 (dd, 1 H, $J_{6a,5}$ 3.5 Hz, $J_{6a,6b}$ 10.8 Hz, H-6a); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 169.54, 159.54 (2 × C-4 of PMB ring), 137.81, 137.16 (2 × C-1 of Bn ether ring) 129.93, 129.91, 128.86, 128.81,128.68, 128.58, 128.30, 128.01 (Ar*C*H), 114.06 (C-3, C-5 of PMB ring), 80.90, 78.49, 77.74 and 77.70 (4 × CH), 75.95, 74.05, 73.86, 73.70, (4 × ArCH_2O), 68.56 (C-6) and 55.64 (OCH_3); MS: (FAB) *m/z* 621.1 [$(M+Na)^+$, 22%], 597.1 [(M), 35 %], *m/z* calcd for $C_{36}H_{38}O_8$ 598.2566: Found *m/z* 598.2518:

2,6-Anhydro-3,7-di-*O*-benzyl-4,5-di-*O*-(*p*-methoxybenzyl)-1-deoxy-*D*-glucohept-1-enitol (14).

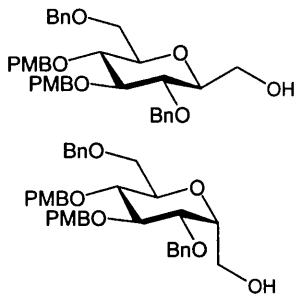


To a solution of **13** (1.07 g, 1.8 mmol) in dry toluene (3.5 mL) and dry THF (1.5 mL), pyridine (26 μL) was added. The mixture was cooled to –45 °C and Tebbe Reagent (3.9 mL, 2 mmol) was added slowly. The mixture was stirred at –40 °C to –45 °C for 1 h then at 0 °C for a further 30 min. It was then cooled to –10 °C to –15 °C and NaOH (1 mL, 15 % w/v) was added. The cooling bath was removed and the reaction mixture was diluted with diethyl ether (50 mL). The inorganic residue was removed by filtration through Celite and the filter cake was washed with excess diethyl ether. The solvents were evaporated off under reduced pressure and the residue was subjected to flash chromatography on silica using diethyl ether–hexane (1:3) as eluent to yield the title compound which was recrystallised from hexane (691 mg, 65.1%).

m.p. 52–54 °C; $[\alpha]^{20}_D$ +42.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.36–7.03 (m, 14 H, ArCH, 2 × H-2 of PMB ring and 2 × H-6 of PMB ring), 6.86–6.77 (m, 4 H, 2 × H-3 of PMB ring, 2 × H-5 of PMB ring), 4.81–4.39 (m, 11 H, H-6, H_2-1', 4 × ArCH_2O), 3.93 (d, 1 H, $J_{3,4}$ 7.2 Hz, H-3), 3.79 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3) and

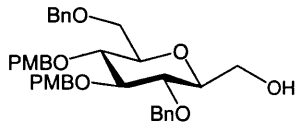
3.74–3.71 (m, 4 H, H-4, H-5, H_2-7); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 159.24, 156.36 (2 × C-4 of PMB ring), 137.93 (2 × C-1 of Bn ether ring), 130.55, 130.29 (2 × C-1 of PMB ring), 129.54, 128.43, 128.35, 127.88, 127.75, 127.62, (Ar*C*H), 113.79, 113.75 (2 × C-3 of PMB ring, 2 × C-5 of PMB ring), 94.61 (C-1), 84.44, 78.98, 78.57, 77.47 (4 × CH), 74.08, 73.50, 73.77 (4 × Ar*C*H_2O), 68.78 (C-7) and 55.25 (OMe); MS: (FAB) *m/z* 597.2 [$(M+H)^+$, 20%], *m/z* calcd for $C_{37}H_{40}O_7$ $[M+H]^+$ 597.2852 found *m/z* 597.2833; Anal.Calcd for $C_{37}H_{40}O_7$ C, 74.47; H, 6.75%. Found: C, 74.30; H, 6.75%:

α and *β*-2,6 di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-*D*-glucopyranosylmethanol (15 and 17).



To compound **14** (570 mg, 0.9 mmol) was added a 1.0 M solution of BH_3-THF in THF (2.9 mL, 2.9 mmol) at 0 °C. It was stirred for 2 h and allowed to heat up to RT. H_2O_2 (0.9 mL, 30%) was added and stirring was continued for 30 min. Water (10 mL) and diethyl ether (20 mL) were added. The organic layer was washed with sat. aq. NaCl (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica using diethyl ether–hexane (2:1) as eluent to give the title compounds as a mixture in a ratio approx 1:2 (587 mg, 73.5%).

β-2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-*D*-glucopyranosylmethanol (17).

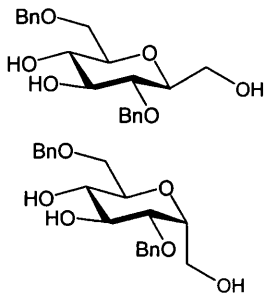


9–BBN (0.5 M in THF, 1.68 mL, 0.8 mmol) was added to **14** (100 mg, 0.2 mmol) at 0 °C and the mixture was stirred for 3 h. H_2O_2 (3 mL, 30%) and 5% aq. KOH were added. The product was extracted into diethyl ether and washed with sat aq. NaCl, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate–hexane (4:6) as eluent to give the title compound as an oil (60 mg, 58%).

$[\alpha]^{20}_D$ +4.5 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.72–7.04 (m, 14 H, 10 × ArC*H*, 2 × H-2 of PMB ring, 2 × H-6 of PMB ring), 6.90–6.80 (m, 4 H, 2 × H-3 of PMB ring, 2 × H-5 PMB ring), 4.88–4.84 (AB, 3 H, J_{AB} 12.0 Hz, 1.5 × ArCH_2O), 4.75, 4.66 (AB, 2 H, J_{AB} 12.0 Hz, ArCH_2O), 4.59, 4.54 (AB, 2 H, J_{AB} 10.5 Hz, ArCH_2O), 4.48 (AB, 1H, J_{AB} 10.5 Hz, 0.5 × ArCH_2O), 3.86 (dd, 1 H, *J* 2.3 Hz, *J* 12.0 Hz, H-1'b), 3.79 (2

× s, 6 H, 2 × OCH_3), 3.74–3.60 (m, 4 H, H-1'a, H-3, H-6a, H-6b), 3.57–3.51 (2 × t, 2 H, H-2, H-4), 3.45 (ddd, 1 H, H-5) and 3.34 (ddd, 1 H, H-1); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 159.41, 159.31 (2 × C-4 of PMB rings), 138.20, 138.07 (2 × C-1 Bn ether ring), 130.93, 130.39 (2 × C-1 of PMB ring), 129.81, 129.58, 128.71, 128.64, 128.23, 128.08, 127.96, (Ar*C*H), 114.09, 114.06 (2 × C-3 of PMB ring, 2 × C-5 of PMB ring), 87.05 (C-3), 79.52 (C-1), 78.94, 78.42, 78.27 (C-5, C-4, C-2), 75.66, 75.34, 75.59, 73.78 (4 × Ar*C*H_2O), 69.44 (C-6), 62.45 (C-1') and 55.64 (O**C**H_3); MS: (FAB) *m/z* 637.2 [(M+Na)$^+$, 80%], 615.2 [(M+H)$^+$, 10%], *m/z* calcd for $C_{37}H_{42}O_8$ [M+H]$^+$ 615.2957 found *m/z* 615.2895; Anal. Calcd for $C_{37}H_{42}O_8$: C, 72.29; H, 6.89%. Found: C, 72.10; H 6.89%:

α and *β*-2,6 di-O-benzyl-*D*-glucopyranosylmethanol (16 and 18).



The mixture of **15** and **17** (87 mg, 0.14 mmol) was dissolved in dichloromethane (10 mL), TFA (1 mL) was added and the mixture was stirred for 20 min. TLC diethyl ether–hexane (7:3) showed that the reaction had gone to completion. Sat. aq. $NaHCO_3$ (50 mL) was added to neutralise the reaction. The products were extracted with dichloromethane (2 × 50 mL), the organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a white solid. It was purified by flash chromatography on silica using ethyl acetate–hexane (8:2) as eluent to give **18** (20.5 mg, 39%)

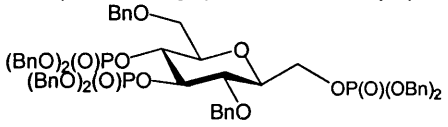
mp 68–70 °C; $[\alpha]^{20}_D$ +6.3 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.38–7.25 (m, 10 H, Ar*C*H), 4.78, 4.72 (AB, 2 H, J_{AB} 11.4 Hz, Ar*C*H_2O), 4.57, 4.54 (AB, 2 H, J_{AB} 12.0 Hz, Ar*C*H_2O), 3.87 (dd, 1 H, *J* 2.3 Hz, H–1'a), 3.70–3.67 (m, 3 H, H-6a, H-6b, H-1'b), 3.64–3.40 (m, 4 H, H-2, H-3, H-4, H-5) and 3.39–3.32 (m, 1 H, H-1); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 138.24, 137.64 (2 × C-1 of Bn ether ring). 128.79, 128.70, 128.26, 128.23, 128.15, 128.03 (Ar*C*H), 79.28, 78.70, 77.62, (3 × CH), 76.99, 75.00 (2 × Ar*C*H_2O), 73.98 (CH), 72.16 (CH), 70.50 (C-6) and 62.39 (C-1'); MS: (FAB) *m/z* 397.2 [(M+Na)$^+$, 73%], 375.2 [(M+H)$^+$, 34%], *m/z* calcd for $C_{21}H_{26}O_6$ [M+H]$^+$ 375.1807 found *m/z* 375.1805:

Further elution gave **16** (22 mg, 41.2%)

$[\alpha]^{20}_D$ +5.6 (*c* 0.17, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.37–7.20 (m, 10 H, Ar*C*H), 4.64, 4.63 (AB, 2 H, J_{AB} 11.7 Hz, Ar*C*H_2O), 4.58, 4.54 (AB, 2 H, J_{AB} 12.0 Hz,

ArCH_2O), 4.17 (ddd, 1 H, $J_{1,2}$ 5.9 Hz, $J_{1,1'a}$ 4.1 Hz, $J_{1,1'b}$ Hz, 9.1 Hz, H-1), 3.90 (dd, 1 H, $J_{1'a,1'b}$ 12.3 Hz, $J_{1'a,1}$ 4.1 Hz, H-1'a), 3.77 (dd, 1 H, $J_{1'b,1'a}$ 12.3 Hz, $J_{1'b,1}$ 9.1 Hz, H-1'b), 3.73–3.63 (m, 5 H, H-3, H-4, H-5, H-6a, H-6b) and 3.60 (dd, 1 H, $J_{2,1}$ 5.9 Hz, $J_{2,3}$ 9.4 Hz, H-2); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 137.60, 137.53 (2 × C-1 of Bn ether ring). 128.82, 128.68, 128.40, 128.21, 128.12, 128.03 (Ar*C*H), 78.53, 77.62 (2 × CH), 74.60, 74.05 (2 × ArCH_2O), 73.53 (CH), 72.14 (C-6), 71.86 (CH), 70.83 (CH) and 58.79 (C-1'); MS: (FAB) *m/z* 397.2 [(M+Na)$^+$, 80%], 375.2 [(M+H)$^+$, 59%], *m/z* calcd for $C_{21}H_{26}O_6$ [M+H]$^+$ 375.1807 found *m/z* 375.1810:

β-2,6-di-O-benzyl-*D*-glucopyranosylmethanol 3,4,1'-tris(dibenzylphosphate) (22)

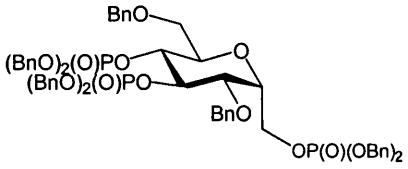


A mixture of bis(benzyloxy)(diisopropylamino) phosphine (276 mg, 0.8 mmol), tetrazole (84 mg, 1.2 mmol) and dry dichloromethane (5 mL) was vigorously stirred at RT for 30 min under N_2, whereupon **18** (50 mg, 0.13 mmol) was added and stirring was continued for 30 min. The mixture was cooled to 0 °C and *m*CPBA (460 mg, 1.6 mmol) was added. The mixture was stirred at RT for 10 min, then was diluted with dichloromethane (50 mL). The solution was washed successively with 10% (w/v) aq. Na_2SO_3 (25 mL), sat. aq. $NaHCO_3$ (25 mL) and sat. aq. NaCl (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The concentrate was purified by flash chromatography on silica using ethyl acetate–hexane (6:4) as eluent to give the title compound as an oil (137 mg, 99.1%).

$[\alpha]^{20}_D$ –5.5 (*c* 1.8, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.46–7.04 (m, 40 H, ArC*H*), 5.09–4.72 (m, 13 H, 6.5 × ArCH_2O), 4.70–4.63 (m, 1 H, H-3), 4.55–4.48 (m, 1 H, H-4), 4.44 (AB, 1 H, J_{AB} 11.1 Hz, 0.5 × ArCH_2O), 4.40 (AB, 1 H, J_{AB} 12.3 Hz, 0.5 × ArCH_2O), 4.30–4.20 (m, 2 H, H-1'a, 0.5 × ArCH_2O), 4.12–4.07 (m, 1 H, H-1'b), 3.79 (dd, 1 H, $J_{6a,5}$ 1.5 Hz, $J_{6a,6b}$ 11.1 Hz, H-6a), 3.66 (dd, 1 H, $J_{6b,5}$ 5.3 Hz, $J_{6b,6a}$ 11.1 Hz, H-6b), 3.65–3.51 (m, 2 H, H-2, H-5) and 3.46–3.43 (m, 1 H, H-1); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 138.24, 137.76 (2 × C-1 Bn ether ring),136.20–135.79 (6 × C-1 of Bn ester rings), 128.73, 128.68, 128.61, 128.54, 128.47, 128.42, 128.38, 128.23, 128.12, 128.09, 127.79, 127.73, 127.62 (Ar*C*H), 74.63, 73.47, 70.16, 70.10, 69.85, 69.66, 69.00 and 66.19 (CH_2); ^{31}P NMR (109 MHz; $CDCl_3$) δ_p 0.02, –0.19, –0.79; MS: (FAB) *m/z* 1177.6

[(M+Na)$^+$, 32%], 1155.6 [(M+H)$^+$, 81%], *m/z* calcd for $C_{63}H_{65}O_{15}P_3$ [M+H]$^+$1155.3614 found *m/z* 1155.3628:

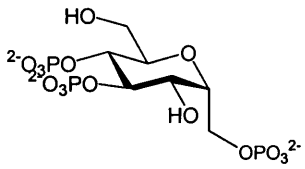
α-2,6-di-O-benzyl-D-glucopyranosylmethanol 3,4,1'-tris(dibenzylphosphate) (21).



A mixture of bis(benzyloxy)(diisopropylamino) phosphine (232 mg, 0.67 mmol), tetrazole (71 mg, 1.00 mmol) and dry dichloromethane (5 mL) was vigorously stirred at RT for 30 min under N_2, whereupon **16** (42 mg, 0.11 mmol) was added and stirring was continued for 30 min. The mixture was cooled to 0 °C and *m*CPBA (460 mg, 1.6 mmol) was added. The mixture was stirred at RT for 10 min, then was diluted with dichloromethane (50 mL). The solution was washed successively with 10% (w/v) aq. Na_2SO_3 (25 mL), sat. aq. $NaHCO_3$ (25 mL) and sat. aq. NaCl (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The concentrate was purified by flash chromatography on silica using ethyl acetate–hexane (6:4) as eluent to give the title compound as an oil (90.5 mg, 78%).

$[\alpha]^{20}_D$ +7.7 (*c* 1.81, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.35–7.16 (m, 40 H, ArC*H*), 5.11–4.86 (m, 12 H, 6 × ArCH_2O), 4.81–4.76 (m, 1 H, H-3), 4.67–4.61 (m, 1 H, H-4), 4.57 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × ArCH_2O), 4.44–4.29 (m, 4 H, H-1'a, 1.5 × ArCH_2O), 4.14–4.04 (m, 2 H, H-1, H-1'b), 3.98–3.94 (m, 1 H, H-5), 3.70–3.64 (m, 2 H, H-6a, H-2) and 3.58 (dd, 1 H, $J_{6b,5}$ 2.9 Hz, $J_{6b,6a}$ 10.8 Hz, H-6b); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 138.15, 137.31 (2 × C-1 of Bn ether ring), 135.91–135.84 (6 × C-1 of Bn ester rings), 128.73, 128.71, 128.65, 128.62, 128.54, 128.41, 128.16, 128.14, 128.13, 127.81, 127.63 (Ar*C*H) 77.66, 77.55 (2 × CH), 73.84, 73.50 (2 × CH_2), 73.35, 71.80, 71.71, (3 × CH), 69.92, 69.76, 69.71, 69.65, 68.14 and 64.10 (CH_2); ^{31}P NMR (162 MHz; $CDCl_3$) δ_P 0.21, –0.594, –1.07; MS; (FAB) *m/z* 1177.6 [(M+Na)$^+$, 32%], 1155.6 [(M+H)$^+$, 85%], *m/z* calcd for $C_{63}H_{65}O_{15}P_3$ [M+H]$^+$1155.3614 found *m/z* 1155.3648:

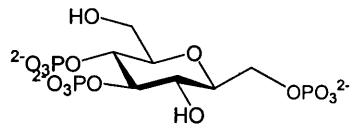
α-D-glucopyranosylmethanol 3,4,1'-trisphosphate (1)



10% Palladium on activated charcoal (50%, 200 mg) was added to a solution of compound **21** (90 mg, 0.08 mmol) in methanol (20 mL) and water (5 mL) and the mixture was hydrogenated at 50 psi at RT for 24 h, after which it was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated under reduced pressure to give a glassy clear solid. The residue was dissolved in de–ionised water and purified by ion exchange chromatography on Q Sepharose Fast Flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of **1** was eluted between 80 and 92%. TEAB fractions containing compound **1**, as judged by phosphate assay, were combined and concentrated to give a residue from which MeOH (3 × 100 mL) was evaporated to give the title trisphosphate as its triethylammonium salt (58 μmole, 72.5%).

$[\alpha]^{20}_{D}$ +19.8 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz; CD_3OD) δ_H 4.35 (q, 1 H, *J* 8.8 Hz, H-3), 4.26–4.12 (m, 3 H, H-1, H-1'a, H-1'b), 4.10–4.05 (q,1 H, *J* 8.8 Hz, H-4), 3.90 (dd, 1 H, $J_{6a,5}$ 4.1 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 3.86–3.79 (m, 2 H, H-2, H-5) and 3.72(dd, 1 H, $J_{6b,5}$ 2.1 Hz, $J_{6b,6a}$ 12.6 Hz, H-6b); ^{31}P NMR (162 MHz; CD_3OD) δ_P 2.70, 2.11, 2.10; (FAB) *m/z* 867.0 [$(2M–H)^-$, 80%], 433.1 [$(M–H)^-$, 100 %], *m/z* calcd for $C_7H_{16}O_{15}P_3$ $[M–H]^-$ 432.9657 found *m/z* 432.9694:

β-D-glucopyranosylmethanol 3,4,1'-trisphosphate (2)

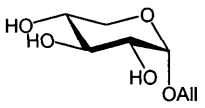


10% Palladium on activated charcoal (50%, 200 mg) was added to a solution of compound **22** (90 mg, 0.08 mmol) in methanol (20 mL) and water (5 mL) and the mixture was hydrogenated at 50 psi at RT for 24 h, after which it was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated under reduced pressure to give a glassy clear solid. The residue was dissolved in de–ionised water and purified by ion exchange chromatography on Q Sepharose Fast Flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of **2** was eluted between 80 and 92%. TEAB fractions containing compound **2**, as judged by phosphate assay, were combined and concentrated to give a residue from which MeOH (3 × 100 mL) was evaporated to give the title trisphosphate as its triethylammonium salt (69 μmole, 86.3%).

$[\alpha]^{20}_{D}$ –3.7 (*c* 1.3, MeOH); ^{1}H NMR (400 MHz; CD_3OD) δ_H 4.22–4.15 (m, 2 H, H-3, H-1'a), 4.09–3.96 (m, 2 H, H-4, H-1'b), 3.86 (dd, 1 H, $J_{6a,5}$ 3.8 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 3.77 (dd, 1 H, $J_{6b,5}$ 1.8 Hz, $J_{6b,6a}$ 12.6 Hz, H-6b), 3.50 (t, 1 H, $J_{2,1}$=2,3 8.8 Hz, H-2), 3.43–3.39 (m, 1 H, H-5) and 3.36–3.29 (m, 1 H, H-1); ^{31}P NMR (162 MHz; CD_3OD) δ_P –5.90, –5.85, –5.38; MS; (FAB) *m/z* 433.1 [$(M–H)^-$, 100 %], *m/z* calcd for $C_7H_{16}O_{15}P_3$ $[M–H]^+$ 432.9657 found *m/z* 432.9684:

7.4 Synthesis of Xylopyranoside trisphosphates

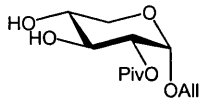
Allyl α-D-xylopyranoside (27)



Allyl alcohol (1 L) and acetyl chloride (6.0 mL) were stirred together for 30 min, after which time D-xylose (100.0 g, 0.67 mol) was added. The mixture was heated at reflux for 16 h and then cooled to room temperature. Solid $NaHCO_3$ (7.00 g) was slowly added, and stirring continued for 30 min. The mixture was then filtered and the filtrate was concentrated under reduced pressure, leaving an off-white solid. The solid was dissolved in minimal ethanol (700 mL) and the solution was kept at –20°C for 24 h. Diisopropyl ether was added immediately before the collection of the title compound over three crops as white fluffy crystals (52.25 g, 41%).

mp 100–103 °C; Lit mp 101–103 °C[76]; $[\alpha]^{20}_D$ +140 (*c* 3.19, $CHCl_3$); Lit $[\alpha]^{20}_D$ +149 (*c* 3.2, $CHCl_3$)[76]; ^{1}H NMR (D_2O; 270 MHz) δ_H 6.01–5.88 (m, 1 H, CH_2CH=CH_2), 5.34 (d, 1 H, ^3J 17.4 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.18 (d, 1 H, ^3J 10.3 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.91 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.20–4.13 (m, 2 H, CH_2CH=CH_2) and 3.76–3.49 (m, 5 H, H-2, H-3, H-4, H-5a, H-5b):

Allyl 2-O-trimethylacetyl α-D-xylopyranoside (34).

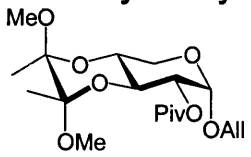


A solution of **27** (10.0 g, 52.6 mmol) in dry pyridine (200 mL) was stirred at –40 °C under N_2. Trimethylacetyl chloride (6.8 mL, 55.2 mmol) was added dropwise over 1 h. The mixture was stirred at –40 °C for a further 90 min. The reaction was quenched with water (100 mL) and was allowed to reach RT and extracted with diethyl ether (100 mL). The organic layer was washed with 1 M HCl (100 mL) and sat. aq. $NaHCO_3$ solution (100 mL), dried ($MgSO_4$), filtered and concentrated to give an oil which was dissolved in hexane and refrigerated at –20 °C. The title compound was collected as crystals over 3 crops (7.6 g, 53%).

mp 76–77 °C; $[\alpha]^{20}_D$ +154 (c 1.7,$CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 5.89–5.77 (m, 1 H, CH_2CH=CH_2), 5.29 (dd, 1 H, ^{2}J 1.5 Hz, ^{3}J 17.0 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.18 (dd, 1 H, ^{2}J 1.5 Hz, ^{3}J 11.7 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.96 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.62 (dd, 1 H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 9.9 Hz, H-2), 4.20–4.15 (m, 1 H, 0.5 × CH_2CH=CH_2), 3.96–3.91 (m, 2 H, H-3, 0.5 × CH_2CH=CH_2), 3.75–3.60 (m, 2 H, H-4, H-5a), 3.60–3.54 (m, 1 H, H-5b), 3.17 (d, 1 H, J 13.3 Hz, OH), 2.97 (d, 1 H, J 9.5 Hz, OH) and 1.22 (s, 9 H, $C(O)C(CH_3)_3$); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 178.60 ($C(O)C(CH_3)_3$), 133.57

($CH_2CH{=}CH_2$), 117.51 ($CH_2CH{=}CH_2$), 95.28 (C-1), 73.07, 72.14, 70.44 (C-2, C-3, C-4), 68.39 ($CH_2CH{=}CH_2$), 61.42 (C-5), 38.89 ($C(O)C(CH_3)_3$) and 27.08 ($C(O)C(CH_3)_3$); MS: (FAB) m/z 571.4 [$(2M+Na)^+$, 62%], 549.4 [$(2M+H)^+$, 50 %], 297.2 [$(M+Na)^+$, 28%], 275.3 [$(M+H)^+$, 20%]; Anal. Calcd for $C_{13}H_{22}O_6$ C, 56.92; H, 8.08%. Found: C, 56.90; H, 8.08%:

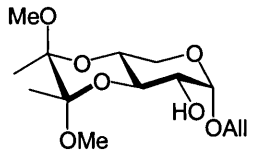
(2'S,3'S) Allyl 3,4-di-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-2-*O*-trimethylacetyl-*α*-*D*-xylopyranoside (35).



A solution of (**34**) (250 mg, 0.91 mmol)l, trimethyl orthoformate (0.39 ml, 3.64 mmol) butane-2,3-dione (0.16 mL, 1.8 mmol) and CSA (20 mg, 0.086 mmol) in methanol (10 mL) was heated under reflux for 3 h, when TLC (diethyl ether) indicated consumption of starting material and showed the presence of one product. The mixture was cooled to RT and triethylamine (1 mL) was added.. The mixture was concentrated under reduced pressure and the syrup obtained was subjected to flash chromatography on silica using dichloromethane–acetone (20:1) as eluent to give the title compound (347 mg, 86%) as an oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.90–5.75 (m, 1 H, $CH_2CH{=}CH_2$), 5.27 (dd, 1 H, ^2J 2.9 Hz, ^3J 17.2 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.15 (dd, 1 H, ^2J 1.6 Hz, ^3J 10.4 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.08 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.70 (dd, 1 H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.4 Hz, H-2), 4.19–4.11 (m ,1 H, 0.5 × $CH_2CH{=}CH_2$), 3.94–3.54 (m, 5 H, H-3, H-4, H-5a, H-5b, 0.5 × $CH_2CH{=}CH_2$), 3.30 (s, 3 H, OCH_3), 3.27 (s, 3 H, OCH_3), 1.29 (s, 3 H, CH_3), 1.20 (s, 3 H, CH_3) and 1.20 (s, 9 H, $C(O)C(CH_3)_3$); ^{13}C NMR (67 MHz; $CDCl_3$) δ_C 177.71, ($C(O)C(CH_3)_3$), 133.70 ($CH_2CH{=}CH_2$), 117.09 ($CH_2CH{=}CH_2$), 99.82, 99.54 (BDA $OCCH_3OCH_3$), 95.48 (C-1), 70.68, 68.41 (2 × CH), 68.40 ($CH_2CH{=}CH_2$), 67.35 (CH), 59.58 (C-5), 49.70, 47.65 (2 × OCH_3), 38.77 ($C(O)C(CH_3)_3$), 27.06 ($C(O)C(CH_3)_3$), and 17.81, 17.55 (2 × CH_3); MS: (FAB) *m/z* 411.2 [$(M+Na)^+$, 55%], 389.2 [$(M+H)^+$ 64%], *m/z* calcd for $C_{19}H_{31}O_8$, $[M+H]^+$ 388.2097 found 388.2062:

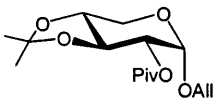
(2'S,3'S) Allyl 3,4-di-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-*α*-*D*-xylopyranoside (28).



A solution of crude **35** (3 g, 7.7 mmol), NaOH (0.61 g, 15 mmol) in methanol (50 ml) was heated under reflux for an hour. The mixture was cooled to room temperature, solid CO_2 was added until the solution was adjusted to pH8. The solvents were removed under reduced pressure and the residue was partitioned between diethyl ether (50 mL) and water (30 mL). The aqueous layer was back-extracted with diethyl ether (50 mL) and the combined organic fractions were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The syrup crystallised as fine needles from light petroleum to give the title compound (800 mg, 34%, over 2 steps).

mp 94–96 °C; Lit mp 98–99 °C[76]; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 5.92–5.84 (m, 1 H, CH_2CH=CH_2), 5.30 (dd, 1 H, ^2J 3.2 Hz, ^3J 17.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.22 (dd, 1 H, ^2J 1.5 Hz, ^3J 10.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.90 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.05–4.01 (m, 2 H, CH_2CH=CH_2), 3.89–3.55 (m, 5 H, H-2, H-3, H-4, H-5a, H-5b) 3.33 (s, 3 H, OCH_3), 3.27 (s, 3 H, OCH_3), 1.35 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3):

Allyl 3,4-*O*-isopropylidene-2-*O*-trimethylacetyl-*α*-*D*-xylopyranoside (36)

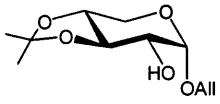


A solution of **34** (5.0 g, 18 mmol) in THF (50 mL) was stirred with p-toluenesulphonic acid (20 mg) and 2-methoxypropene (3.49 mL, 36 mmol) at RT for 30 min under N_2. TLC (ethyl acetate) indicated consumption of starting material and showed the presence of one product. The mixture was diluted with diethyl ether (50 mL) and washed with sat. aq. $NaHCO_3$ (30 mL). The organic layer was dried ($MgSO_4$) and filtered, a few drops of Et_3N were added before the organic layer was concentrated under reduced pressure. The residue was used directly in the next step without purification. A small sample was purified for analysis by flash chromatography on silica using diethyl ether–hexane (1:7) as eluent.

$[\alpha]_D^{20}$ +143 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.90–5.80 (m, 1 H, CH_2CH=CH_2), 5.29 (d, 1 H, ^{3}J 17.3 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 5.19 (d, 1 H, ^{3}J 10.2 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 5.16 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.82 (dd, 1 H, $J_{2,1}$ 3.8 Hz, $J_{2,3}$ 9.0 H-2), 4.21–4.16 (m, 1 H, 0.5 × CH_2CH=CH_2), 3.96–3.90 (m, 3 H, H-3, H-5a, 0.5 ×

$CH_2CH{=}CH_2$), 3.76 (d, 1 H, ^3J 10.2 Hz, H-5b), 3.54–3.48 (m, 1 H, H-4), 1.46 (s, 6 H, 2 × isopropylidene CH_3) and 1.23 (s, 9 H, $C(O)C(CH_3)_3$); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 177.96 ($C(O)C(CH_3)_3$), 133.50 ($CH_2CH{=}CH_2$), 117.83 ($CH_2CH{=}CH_2$), 110.86 (isopropylidene $C(CH_3)_2$), 95.10 (C-1), 75.75 (C-2), 74.12 (C-3), 72.88 (C-4), 68.96 ($CH_2CH{=}CH_2$), 61.27 (C-5), 38.77 ($C(O)C(CH_3)_3$) and 27.10, 26.81, 26.54 (2 × isopropylidene CH_3, $C(O)C(CH_3)_3$); MS: (FAB) m/z 315 [$(M+H)^+$, 49%]; Anal. Calcd for $C_{16}H_{26}O_6$ C, 61.13; H, 8.34%. Found: C, 61.00; H, 8.29%:

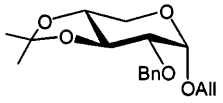
Allyl 3,4-*O*-isopropylidene-*α*-*D*-xylopyranoside (37).



A solution of crude **36** (5.72 g, 18.0 mmol) and NaOH (1.46 g, 36 mmol) in MeOH (100 mL) was heated under reflux for 1 h. The mixture was cooled to RT and neutralised by careful addition of solid CO_2. The solvents were evaporated off under reduced pressure and the residue was partitioned between diethyl ether(100 mL) and water (50 mL). The aqueous layer was back-extracted with diethyl ether (100 mL) and the combined organic fractions were dried ($MgSO_4$). A few drops of Et_3N were added before the organic layer was concentrated under reduced pressure. The residue was used directly in the next step without purification. For analytical purposes, a small sample was purified by flash chromatography on silica using diethyl ether–hexane(1:1) as eluent.

$[\alpha]_D^{20}$ +133 (c 0.4, $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$) δ_H 5.97–5.87 (m, 1 H, $CH_2CH{=}CH_2$), 5.32 (d, 1 H, ^3J 17.3 Hz, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.24 (d, 1 H, ^3J 10.2 Hz, $CH_2CH{=}CH_{cis}CH_{trans}$), 4.95 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.28–4.23 (m, 1 H, 0.5 × $CH_2CH{=}CH_2$), 4.07–4.02 (m, 1 H, 0.5 × $CH_2CH{=}CH_2$), 3.94 (t, 1 H, $J_{3,4}$ 9.7 Hz, $J_{3,2}$ 4.4 Hz, H-3), 3.83 (dd, 1 H, $J_{2,1}$ 3.8 Hz, $J_{2,3}$ 4.4 Hz, H-2), 3.74–3.66 (m, 2 H, H-5a, H-5b), 3.44–3.38 (m, 1 H, H-4), 2.43 (d, 1 H, J 10.8 Hz, 2-OH) and 1.46, 1.45 (2s, 6 H, 2 × isopropylidene CH_3); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 133.35 ($CH_2CH{=}CH_2$), 118.07 ($CH_2CH{=}CH_2$), 110.70 (isopropylidene $C(CH_3)_2$), 97.50 (C-1), 79.3 (C-2), 73.6, 71.9 (C-4, C-3, C-2), 68.84 ($CH_2CH{=}CH_2$), 61.57 (C-5) and 26.73, 26.40 (2 × isopropylidene CH_3); MS: (FAB) m/z 231 [$(M+H)^+$, 100%]; Anal. Calcd for for $C_{11}H_{18}O_5$: C, 57.38; H, 7.94%. Found C, 57.10; H, 7.94%:

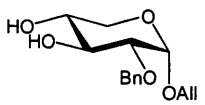
Allyl 2-O-benzyl-3,4-O-isopropylidene-α-D-xylopyranoside (38).



A solution of crude **37** (4.18 g, 18 mmol) in dry DMF (25 mL) was stirred at 0 °C with NaH (760 mg of an 60% w/w dispersion in mineral oil, 19 mmol) and benzyl bromide (2.17 mL, 19 mmol) was added slowly under N_2. The mixture was stirred at RT for 90 min, after which time TLC (diethyl ether–hexane 1:2) showed complete consumption of starting material. Water (25 mL) was added carefully and stirring continued for 1 h. The solvents were evaporated under reduced pressure and the residue was dissolved in diethyl ether (50 mL). The extract was washed with water (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The product of this reaction was used directly in the next step without purification. A small sample was purified for analysis by flash chromatography on silica using diethyl ether–hexane (1:9) as eluent.

$[\alpha]_D^{20}$ +30.6 (c 0.5, $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$) δ_H 7.39–7.24 (m, 5H, ArCH), 5.96–5.86 (m, 1 H, $CH_2CH{=}CH_2$), 5.34 (d, 1 H, ^3J 17.3 Hz, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.21 (d, 1 H, ^3J 10.2 Hz, 0.5 × $CH_2CH{=}CH_{cis}CH_{trans}$), 4.86–4.83 (m, 2 H, H-1, 0.5 × OCH_2Ar), 4.64 (AB, 1 H, J_{AB} 12.3 Hz, 0.5 × OCH_2Ar), 4.20–4.15 (m, 1 H, 0.5 × $CH_2CH{=}CH_2$), 4.00–3.91 (m, 2 H, H-3, 0.5 × $CH_2CH{=}CH_2$), 3.86 (dd, 1 H, $J_{5a,5b}$ 9.7 Hz, $J_{5a,4}$ 4.7 Hz, H-5a), 3.73 (dd, 1 H, $J_{5b,5a}$ 9.7 Hz, H-5b), 3.65 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 10.2 Hz, H-2), 3.41–3.35 (m, 1 H, H-4) and 1.47, 1.46 (2s, 6 H, 2 × isopropylidene CH_3); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 138.05 (C-1 of benzyl ether ring), 133.69 ($CH_2CH{=}CH_2$), 128.26, 127.86, 127.62 (ArCH), 117.80 ($CH_2CH{=}CH_2$), 110.54 (isopropylidene $C(CH_3)_2$), 96.24 (C-1), 77.91, 77.90, 74.10 (C-4, C-3, C-2), 71.8 (OCH_2Ar), 68.44 ($CH_2CH{=}CH_2$), 61.09 (C-5) and 26.82, 26.43 (2 × isopropylidene CH_3); MS: (FAB) m/z 321 [$(M+H)^+$, 14%]; Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55%. Found C, 67.40%; H, 7.53%:

Allyl 2-O-benzyl-α-D-xylopyranoside (31)

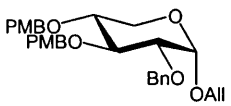


A solution of **38** (5.86 g, 18 mmol) in MeOH (25 mL) was stirred with 1 M HCl (2.5 mL) for 30 min. TLC (diethyl ether-hexane 4:1) indicated consumption of starting material. Solid $NaHCO_3$ was added until the mixture was neutral. The solvents were evaporated off and the residue was partitioned between dichloromethane (50 mL) and water (25 mL). The aqueous layer

was back extracted with dichloromethane (100 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The syrup obtained was subjected to flash chromatography on silica using diethyl ether–hexane (4:1 then 1:0) as eluent to give the title compound as a colourless syrup (3.87 g, 76% over the previous 4 steps) which slowly crystallised.

mp 46–47 °C (from diisopropyl ether); Lit oil[76]. $[\alpha]_D^{20}$ +115 (c 2.3, $CHCl_3$); Lit $[\alpha]_D^{20}$ +113 (c 2.3, $CHCl_3$)[76]; ^{1}H NMR ($CDCl_3$; 270 MHz) δ_H 7.36–7.24 (m, 5 H, ArCH), 5.95–5.81 (m, 1 H, $CH_2CH{=}CH_2$), 5.31 (dd, 1 H, ^2J 1.6 Hz, ^3J 17.2 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.18 (dd, 1 H, ^2J 1.5 Hz, ^3J 10.4 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 4.78 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.67, 4.61 (AB, 2 H, J_{AB} 11.9 Hz, $ArCH_2O$), 4.17–4.06 (m, 1 H, 0.5 × $CH_2CH{=}CH_2$), 3.92–3.83 (m, 2 H, 0.5 × $CH_2CH{=}CH_2$,H-3), 3.62–3.43 (m, 5 H, H-4, H-5a, H-5b, 2 × OH) and 3.31 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 9.5 Hz, H-2):

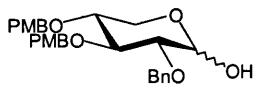
Allyl 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-α-*D*-xylopyranose (32).



A solution of **31** (4.1 g, 14 mmol) in dry DMF (60 mL) was stirred with NaH (1.58 g of an 60% w/w dispersion in mineral oil, 40 mmol) and *p*-methoxybenzyl chloride (8.0 mL, 59 mmol) at rt for 3 h. Methanol (30 mL) was added and stirring continued for 15 min. The solvents were concentrated under reduced pressure and the residue was dissolved in diethyl ether. The organic solution was washed with water, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica using hexane–ethyl acetate (19:1) as eluent to give the title compound as an oil.

^{1}H NMR ($CDCl_3$; 270 MHz) δ_H 7.37–7.22 (m, 9 H, ArCH, 2 × H-2 of PMB ring, 2 × H-6 of PMB ring), 6.87–6.83 (m, 4 H, 2 × H-3 of PMB ring, 2 × H-5 of PMB ring), 5.97–5.85 (m, 1 H, $CH_2CH{=}CH_2$), 5.31 (dd, 1 H, ^2J 1.5 Hz, ^3J 17.2 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.03 (d, 1 H, ^3J 10.3 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 4.84, 4.79 (AB, 2 H, J_{AB} 10.4 Hz, $ArCH_2O$), 4.71 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.77, 4.64 (AB, 2 H, J_{AB} 12.2 Hz, $ArCH_2O$), 4.68, 4.54 (AB, 2 H, J_{AB} 11.2 Hz, $ArCH_2O$), 4.18–4.11 (m, 1 H, 0.5 × $CH_2CH{=}CH_2$), 4.00–3.96 (m, 1 H, 0.5 × $CH_2CH{=}CH_2$), 3.93–3.86 (m, 1 H, H-3), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.53–3.49 (m, 3 H, H-4, H-5a, H-5b) and 3.43 (dd, 1 H, $J_{2,1}$ 3.4 Hz, $J_{2,3}$ 9.5 Hz, H-2):

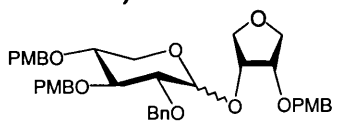
2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-α-*D*-xylopyranose (33).



A solution of allyl 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-α-*D*-xylopyranoside **32** (6.87 g, 13.2 mmol) in MeOH (60 mL) was cooled to 0°C and $PdCl_2$ (0.47 g, 2.64 mmol) was added. The flask was fitted with a drying tube, the cooling bath removed and the reaction mixture was stirred vigorously for 4 h, after which time TLC (diethyl ether–hexane 3:2) indicated almost complete conversion of starting material into a product. The reaction mixture was quenched with triethylamine and filtered through a Celite pad. The filtrate was concentrated and the dark brown residue was subjected to flash chromatography on silica using diethyl ether–hexane (3:2) as eluent to yield the title compound (mixture of α and β anomers) as a white solid (5.72 g, 90%).

^{1}H NMR ($CDCl_3$; 400 MHz:D_2O exchange) δ_H 7.34–7.23 (m, 9 H, ArCH), 6.87–6.83 (m, 4 H, 2 × H-3 of PMB ring, 2 × H-5 of PMB ring), 5.09 (d, 0.5 H, $J_{1,2}$ 3.7 Hz, H-1α), 4.89–4.54 (m, 6.5 H, H-1β, 3 × $ArCH_2O$), 3.82 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.92–3.50 (m, 3.5 H, H-3, H-4α, H-5a, H-5b), 3.45 (dd, 0.5 H, $J_{2\alpha,1\alpha}$ 3.7 Hz, $J_{2\alpha,3\alpha}$ 8.9 Hz, H-2α) and 3.30–3.21 (m, 1 H, H-2β, H-4β):

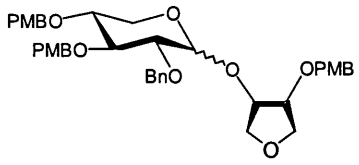
1-*O*-[(3'S,4'R)-3-(*p*-methoxybenzyloxy)tetrahydrofuran-4-yl] 2-*O*-benzyl 3,4-di-*O*-(*p*-methoxybenzyl)-α and β-*D*-xylopyranosides (42 and 43).



To a stirred mixture of **33** (2.0 g, 4.6 mmol) and 1H-tetrazole (440 mg, 6.25 mmol) in dichloromethane (10 mL) was added bis(methoxy)(diethylamino)phosphine (0.9 mL, 5.4 mmol). After 20 min, TLC (ethyl acetate–toluene 1:4) indicated complete conversion into product. The reaction mixture was partitioned between diethyl ether (100 mL) and water (75 mL). The resulting ethereal layer was washed with brine (75 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give crude 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-D-xylopyranosyl dimethyl phosphite **39** [63] as a colourless oil (2.38 g), which was used without further purification. A solution of the dimethyl phosphite (2.38 g), (+)-(3R,4S)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol **40** [57] (747 mg, 3.3 mmol) and 4 Å molecular sieves (3 g) in dioxane–toluene (21 mL:7 mL) was stirred for 2 h under N_2. Zinc chloride (679 mg, 4.99 mmol) and silver perchlorate (2.04 g, 9.88 mmol) were added to the reaction mixture which was stirred in the dark for a further 2 h, TLC

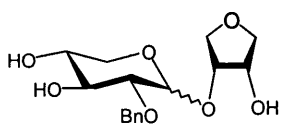
(toluene–ethyl acetate 4:1) showed consumption of the starting material. $NaHCO_3$ (3 g), ethyl acetate (100 mL) and water (75 mL) were added to the mixture and it was stirred vigorously for a further 30 min. The mixture was filtered through a Celite pad and the organic layer was washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The oil obtained was subjected to flash chromatography on silica using ethyl acetate–hexane (7:3) to give an anomeric mixture of the title compounds which could not be separated at this stage (1.60 g, 70%):

1-*O*-[(3'R,4'S)-3-(*p*-methoxybenzyloxy)tetrahydrofuran-4-yl] 2-*O*-benzyl 3,4-bis-*O*-(*p*-methoxybenzyl)-α and β-*D*-xylopyranosides (44 and 45).



To a stirred mixture of **33** (2.0 g, 4.6 mmol) and 1*H*-tetrazole (440 mg, 6.25 mmol) in dichloromethane (10 mL) was added bis(methoxy)(diethylamino)phosphine (0.9 mL, 5.4 mmol). After 20 min, TLC (ethyl acetate–toluene 1:4) indicated complete conversion into product. The reaction mixture was partitioned between diethyl ether (100 mL) and water (75 mL). The resulting ethereal layer was washed with brine (75 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give crude 2-O-benzyl-3,4-bis-O-(p-methoxybenzyl)-d-xylopyranosyl dimethyl phosphite as a colourless oil (2.38 g), which was used without further purification. A solution of the dimethyl phosphite (2.38 g), (–)-(3S,4R)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol (**41**) [57] (747 mg, 3.3 mmol) and 4 Å molecular sieves (3 g) in dioxane–toluene (21 mL:7 mL) was stirred for 2 h under N_2. Zinc chloride (679 mg, 4.99 mmol) and silver perchlorate (2.04 g, 9.88 mmol) were added to the reaction mixture which was stirred in the dark for a further 2 h, TLC (toluene–ethyl acetate 4:1) showed consumption of the starting material. $NaHCO_3$ (3 g), ethyl acetate (100 mL) and water (75 mL) were added to the mixture and it was stirred vigorously for a further 30 min. The mixture was filtered through a Celite pad and the organic layer was washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The oil obtained was subjected to flash chromatography on silica using ethyl acetate–hexane (7:3) to give an anomeric mixture of the title compounds which could not be separated at this stage (1.75 g, 76%):

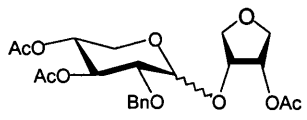
1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl]-2-*O*-benzyl-α-*D*-xylopyranoside (46) and 1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-β-*D*-xylopyranoside (47).



A solution of the mixture of **42** and **43** (1.60 g, 2.32 mmol) and TFA (2 mL) in dichloromethane (20 mL) was stirred for 20 min, TLC (dichloromethane–acetone 10:1) showed consumption of starting material. Sat. aq. $NaHCO_3$ was added to neutralise the reaction. The mixture was extracted with ethyl acetate (4 × 100 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The oil obtained was subjected to flash chromatography on silica using ethyl acetate–methanol (20:1) to give a mixture of the title compounds (341 mg, 45%). **46** (261 mg, 35 %) Crystallised from ethyl acetate–hexane (261 mg, 34%).

mp 119–120 °C (EtOAc–hexane); $[\alpha]^{20}_D$ +87.2 (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz;D_2O exchange) δ_H 7.37–7.26 (m, 5 H, ArCH), 4.77 (d, 1 H, $J_{1,2}$ 2.9 Hz, H-1), 4.75, 4.71 (AB, 2 H, J_{AB} 11.7 Hz, $ArCH_2O$), 4.26–4.25 (m, 1 H, H-3'), 4.11–4.07 (m, 1 H, H-4'), 3.96 (dd, 1 H, $J_{5'a,4'}$ 5.9 Hz, $J_{5'a,5'b}$ 9.4 Hz, H-5'a), 3.91–3.88 (m, 2 H, H-3, H-2'a), 3.80 (dd, 1 H, $J_{5'b,4'}$ 4.4 Hz, $J_{5'b,5'a}$ 9.4 Hz, H-5'b), 3.72–3.68 (m, 1 H, H-2'b), 3.65–3.51 (m, 3 H, H-4, H-5a, H-5b) and 3.35 (dd, 1 H, $J_{2,3}$ 8.8 Hz, $J_{2,1}$ 2.9 Hz, H-2); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.34 (C-1 of benzyl ether ring), 128.92, 128.73, 128.68 (3 × Ar), 98.82 (C-1), 79.40, 79.00 (2 × CH), 74.59 (CH_2), 73.78 (CH), 73.13 (CH_2), 71.11 (CH), 71.11 ($ArCH_2O$), 70.28 (CH) and 62.41 (C-5); MS: (FAB) m/z 349.2 [$(M+Na)^+$, 22%], 327.2 [$(M+H)^+$, 32%]; Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.79%. Found C, 58.80; H, 6.76%:

1-*O*-[(3S,4R)-3-acetoxytetrahydrofuran-4-yl] 3,4-di-*O*-acetyl-2-*O*-benzyl-α-*D*-xylopyranoside (50) and 1-*O*-[(3'S,4'R)-3-acetoxytetrahydrofuran-4-yl] 3,4-di-*O*-acetyl-2-*O*-benzyl-β-*D*-xylopyranoside (51).



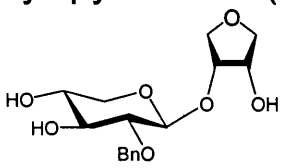
The mixture of compounds (**46**, **47**) (90 mg, 0.27 mmol) was dissolved in acetic anhydride (0.16 mL, 1.65 mmol) and pyridine (1 mL) and stirred overnight. The mixture was concentrated under reduced pressure from toluene (3 × 10 mL). The products were purified by flash chromatography on silica using diethyl ether–hexane (5:2) as eluent to give **50** (13 mg, 10%).

$[\alpha]^{20}_D$ +63.8 (*c* 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.35–7.28 (m, 5 H, ArC*H*), 5.43 (t, 1 H, $J_{3,2}$=$J_{3,4}$ 9.7 Hz, H-3), 5.28–5.25 (m, 1 H, H-3'), 4.92 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.90–4.84 (m, 1 H, H-4), 4.68, 4.51 (AB, 2 H, J_{AB} 12.3 Hz, ArCH_2O), 4.35–4.30 (m, 1 H, H-4'), 4.06 (dd, 1 H, $J_{2'a,3'}$ 5.8 Hz, $J_{2'a,2'b'}$ 9.9 Hz, H-2 'a), 3.97 (dd, 1 H, $J_{5'a,4'}$ 6.1 Hz, $J_{5'a,5'b}$ 9.1 Hz, H-5'a), 3.90–3.81 (m, 2 H, H-2'b, H-5'b) 3.75 (dd, 1 H, $J_{5a,4}$ 5.9 Hz, $J_{5a,5b}$ 10.8 Hz, H-5a), 3.54 (t, 1 H, $J_{5b,4}$=$J_{5b,5a}$ 10.8 Hz, H-5b), 3.46(dd, 1 H, $J_{2,3}$ 9.7 Hz, $J_{2,1}$ 3.2 Hz, H-2), 2.02 (s, 3 H, CH_3CO), 1.99 (s, 3 H, CH_3CO) and 1.91 (s, 3 H, CH_3CO); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 171.07, 170.22 (3 × O*C*OCH$_3$), 137.99 (C-1 of benzyl ether ring), 128.58, 128.03, 127.87 (3 × Ar), 97.49 (C-1), 77.17, 76.13 (2 × CH), 73.28, 71.34, 71.18 (2 × CH_2, 2 × Ar*C*H$_2$O), 70.93, 70.77, 69.72 (3 × CH), 59.38 (C-5), 21.36, 21.20, 21.09 (3 × CH_3*C*O); MS: (FAB) *m/z* 475.2 [(M+Na)$^+$, 15%]:

Further elution gave **51** (81 mg, 65%) which crystallised from diethyl ether–hexane.

mp 108–110 °C; $[\alpha]^{20}_D$ –21.9 (*c* 0.91, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 8.02–7.25 (m, 5 H, ArC*H*), 5.26–5.22 (m, 1 H, H-3'), 5.14 (t, 1 H, $J_{3,2}$=$J_{3,4}$ 9.4 Hz, H-3), 4.92–4.85 (m, 1 H, H-4), 4.80, 4.61 (AB, 2 H, J_{AB} 12.3 Hz, ArCH_2O), 4.50–4.44 (m, 2 H, H-1, H-4'), 4.09–3.99 (m, 3 H, H-5a, H-2'a, H-5'a), 3.88–3.84 (m, 2 H, H-2'b, H-5'b), 3.40 (dd, 1 H, $J_{2,3}$ 9.4 Hz, $J_{2,1}$ 7.0 Hz, H-2), 3.28 (dd, 1 H, $J_{5b,4}$ 9.4 Hz, $J_{5b,5a}$ 11.7 Hz, H-5b), 2.11 (s, 3 H, CH_3CO), 2.01 (s, 3 H, CH_3CO) and 1.95 (s, 3 H, CH_3CO); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 170.06, 169.75, 169.66 (3 × O*C*OCH$_3$), 137.40 (C-1 of benzyl ether ring), 128.17, 127.76, 127.56 (3 × Ar), 102.96 (C-1), 78.07, 77.05 (2 × CH), 74.22 (CH_2), 72.54, 72.06 (2 × CH), 70.18, 70.06 (2 × Ar*C*H$_2$O), 69.10 (CH), 62.44 (C-5), 20.97, 20.76, 20.73 (3 × CH_3*C*O); MS: (FAB) *m/z* 475.1 [(M+Na)$^+$, 10%], 451.1 [(M+H)$^+$, 9%], *m/z* calcd for $C_{22}H_{28}O_{10}$, [M+H]$^+$ 453.1760 found 453.1729:

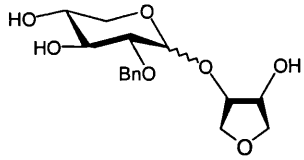
1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*β*-*D*-xylopyranoside (47).



A solution of **51** (50 mg, 0.11 mmol) and concentrated ammonia (0.75 mL) in methanol (4 mL) was stirred at rt overnight. The mixture was concentrated under reduced pressure from dichloromethane (3 × 10 mL). The products were purified by flash chromatography on silica using ethyl acetate–methanol (20:1) as eluent to give (30 mg, 83%).

$[\alpha]^{20}_D$ +8.33 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.34–7.26 (m, 5 H, ArC*H*), 4.85, 4.65 (AB, 2 H, J_{AB} 11.4 Hz, ArCH_2O), 4.45 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.25–4.19 (m, 2 H, H-4', H-3'), 3.98–3.94 (m, 3 H, H-5a, H-2'a, H-5'a), 3.80–3.77 (m, 2 H, H-2'b, H-5'b), 3.63–3.62 (m, 1 H, H-4), 3.52–3.45 (m, 1 H, H-3), 3.30–3.23 (m, 4 H, H-2, H-5b, 2 × OH) and 3.18 (broad s, 1H, OH); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.96 (C-1 of benzyl ether ring), 128.84, 128.36, 128.22 (Ar), 104.02 (C-1), 81.43, 80.63 and 75.75 (3 × CH), 74.96, 73.29 (CH_2), 71.04 (CH), 70.19 (Ar*C*H_2O), 69.51 (CH) and 65.60 (C-5); MS: (FAB) *m/z* 349.1 $[(M+Na)^+$, 18%], 327.1 $[(M+H)^+$, 20%], *m/z* calcd for $C_{16}H_{22}O_7$, $[M+H]^+$327.1443 found 327.1455:

1-*O*-[(3'R,4'S)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*α*-*D*-xylopyranoside (48) and 1-*O*-[(3'R,4'S)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*β*-*D*-xylopyranoside (49).



A solution of the mixture of **44** and **45** (1.75 g, 2.50 mmol) and TFA (2 mL) in dichloromethane (20 mL) was stirred for 20 min, TLC (dichloromethane–acetone 10:1) showed consumption of starting material. Sat. aq. $NaHCO_3$ was added to neutralise the reaction. The mixture was extracted with ethyl acetate (4 × 100 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The oil obtained was subjected to flash chromatography on silica using ethyl acetate–methanol (20:1) to give the title compound **48** which was crystallised from ethyl acetate–hexane (145 mg, 18%).

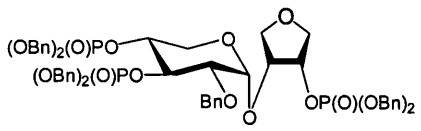
mp 144–145 °C; $[\alpha]^{20}_D$ +132 (*c* 0.2, $CDCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.35–7.25 (m, 5 H, ArC*H*), 4.74 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.66, 4.57 (AB, 2 H, J_{AB} 11.4 Hz, ArCH_2O), 4.25–4.22 (m, 1 H, H-3'), 4.16–4.12 (m, 1 H, H-4'), 3.99–3.89 (m, 2 H, H-3, H-5'a), 3.85 (dd, 1 H, $J_{2'b,3'}$ 5.6 Hz, $J_{2'b,2'a}$ 9.4 Hz, H-2'a), 3.76–3.72 (m, 2 H, H-2'b, H-5'b), 3.63–3.46 (m, 3 H, H-4, H-5a, H-5b) and 3.33 (dd, 1 H, $J_{2,3}$ 9.4 Hz, $J_{2,1}$ 3.5 Hz, H-

2); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 137.28 (C-1 of benzyl ether ring), 128.63, 128.43 (ArCH), 95.08 (C-1), 79.44, 76.53 (2 × CH), 74.79 (CH_2), 73.49 (CH), 72.57 (CH_2), 71.40, 69.63 (2 × CH), 68.89 (Ar*C*H_2O) and 61.85 (C-5); MS: (FAB) *m/z* 349.2 [(M+Na)$^+$, 30%], 327.2 [(M+H)$^+$, 28%]; Anal. Calcd for $C_{16}H_{22}O_7$ C, 58.88; H, 6.79%. Found: C, 58.70; H, 6.79%:

Further elution gave compound **49** which was crystallised from EtOAc–hexane (155 mg, 19%);

mp 166–167 °C; $[\alpha]^{20}_D$ –28.3 (*c* 0.4, MeOH); ^1H NMR (CD_3OD; 400 MHz) δ_H 7.43–7.27 (m, 5 H, ArC*H*), 4.95, 4.81 (AB, 2 H, J_{AB} 11.1 Hz, ArCH_2O), 4.55 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.31–4.23 (m, 2 H, H-2'a and H-5'a), 3.98–3.94 (m, 1 H, H-3' or H-4'), 3.90–3.86 (m, 2 H, H-3, H-2'b or H-5'b), 3.80–3.76 (m, 1 H, H-3' or H-4'), 3.63 (dd, 1 H, *J* 4.1 Hz, J_{gem} 9.1 Hz, H-2'b or H-5'b), 3.57–3.46 (m, 2 H, H-4, H-5a) and 3.30–3.21 (m, 2 H, H-2, H-5b); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 140.21 (C-1 of benzyl ether ring), 129.70, 129.54 and 128.96 (Ar), 105.10 (C-1), 83.02, 80.18, 78.02 (3 × CH), 75.98, 73.66 (2 × CH_2), 72.23 (Ar*C*H_2O), 72.13, 71.60 (2 × CH) and 67.17 (C-5); MS: (FAB) *m/z* 349.2 [(M+Na)$^+$, 24%], 327.2 [(M+H)$^+$, 28%]; Anal. Calcd for $C_{16}H_{22}O_7$ requires C, 58.88; H, 6.79%. Found: C, 58.50; H, 6.80%:

1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*α*-*D*-xylopyranoside 3,4,3'-tris(dibenzylphosphate) (52).

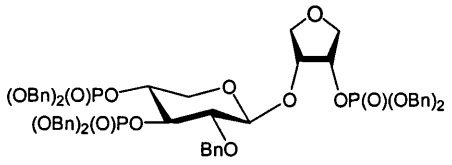


A mixture of bis(benzyloxy)(diisopropylamino) phosphine (518 mg, 1.50 mmol), 1*H*-tetrazole (158 mg, 2.25 mmol) and dry dichloromethane (5 mL) was vigorously stirred at rt for 30 min under N_2, whereupon triol **46** (83 mg, 0.25 mmol) was added and stirring was continued for 30 min. The mixture was cooled to –78 °C and *m*CPBA (863 mg, 60%, 3.0 mmol) was added. The mixture was stirred at rt for 10 min and was diluted with dichloromethane (50 mL). The solution was washed successively with 10% (w/v) aq. Na_2SO_3, sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate–hexane (8:2) as eluent to give the title compound as an oil (279 mg, 99%).

$[\alpha]^{20}_D$ +0.3 (c 1.9, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.39–7.04 (m, 35 H, ArCH), 5.06–4.66 (m, 16 H, H-1, H-3, H-3', 6.5 × ArCH_2O), 4.47–4.31 (m, 2 H, H-4, 0.5 ×

$ArCH_2O$), 4.12–4.08 (m, 1 H, H-4'), 3.96–3.84 (m, 4 H, H-5a, H-5b, H-2'a, H-5'a), 3.79 (dd, 1 H, $J_{5'b,4'}$ 5.3 Hz, $J_{5'b,5'a}$ 9.4 Hz, H-5'b) and 3.49–3.43 (m, 2 H, H-2, H-2'b); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 137.59 (C-1 of benzyl ether ring), 136.02–135.50 (C-1 of benzyl ester rings), 128.50, 128.50, 128.48, 128.41, 128.61, 128.22, 128.13, 128.06, 127.94, 127.88, 127.71, 127.60, 127.49 (ArCH), 95.68 (C-1), 77.09, 75.35, 75.26, 74.03 and 73.93 (5 × CH), 71.97, 69.99 (2 × CH_2), 68.91– 69.71 ($ArCH_2O$) and 59.69 (C-5); ^{31}P NMR ($CDCl_3$; 162 MHz). δ_P –0.70, –0.62 and 0.11; MS: (FAB) m/z 1128.9 $[(M+Na)^+$, 60%], 1107.0 $[(M+H)^+$, 98%], m/z calcd for $C_{58}H_{61}O_{16}P_3$ $[M+H]^+$ 1107.3250 found m/z 1107.3233:

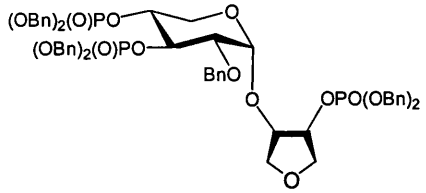
1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*β*-*D*-xylopyranoside 3,4,3'-tris(dibenzylphosphate) (53).



A mixture of bis(benzyloxy)(diisopropylamino) phosphine (191 mg, 0.55 mmol), 1*H*-tetrazole (58 mg, 0.83 mmol) and dry dichloromethane (5 mL) was vigorously stirred at rt for 30 min under N_2, whereupon triol **47** (30 mg, 0.09 mmol) was added and stirring was continued for 30 min. The mixture was cooled to –78 °C and *m*CPBA (317 mg, 60%, 1.1 mmol) was added. The mixture was stirred at RT for 10 min, then was diluted with dichloromethane (50 mL). The solution was washed successively with 10% (w/v) aq. Na_2SO_3, sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate–hexane (8:2) as eluent to give the title compound as an oil (89 mg, 87%). $[\alpha]^{20}_D$ –10.4 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.34–7.02 (m, 35 H, ArC*H*), 5.14–4.75 (m, 14 H, H-3', 6.5 × ArCH_2O), 4.58–4.49 (m, 2 H, H-3, 0.5 × ArCH_2O), 4.39 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.35–4.31 (m, 1 H, H-4'), 4.30–4.23 (m, 1 H, H-4), 4.19 (m, 1 H, H-5a), 3.93–3.85 (m, 3 H, H-5'a, H-2'a, H-2'b), 3.74 (dd, 1 H, $J_{5'b,4'}$ 6.5 Hz, H-5'b) and 3.28–3.23 (m, 2 H, H-2, H-5b); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.89 (C-1 of benzyl ether ring), 136.64–135.12 (C-1 of benzyl ester ring), 128.83, 128.80, 128.75, 128.61, 128.59, 128.46, 128.23, 128.21, 128.12, 128.03, 127.97, 127.91, 127.81 (Ar), 103.09 (C-1), 79.55, 79.26, (2 × CH), 76.40 (CH_2), 74.58, 73.86, 71.36 (3 × CH), 70.10 (CH_2), 68.87–70.04 (7 × ArCH_2O) and 63.42 (C-5); ^{31}P NMR ($CDCl_3$; 162

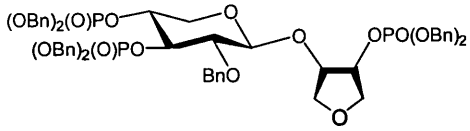
MHz) δ_p –0.76, –0.60 and –0.43; MS: (FAB) *m/z* 1129.1 [$(M+Na)^+$, 60%], 1107.2 [$(M+H)^+$, 83%], *m/z* calcd for $C_{58}H_{61}O_{16}P_3$ [$M+H]^+$ 1107.3238 found *m/z* 1107.3238:

1-*O*-[(3'R,4'S)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*α*-*D*-xylopyranoside 3,4,3'-tris(dibenzylphosphate)(54).



A mixture of bis(benzyloxy)(diisopropylamino) phosphine (539 mg, 1.56 mmol), 1*H*-tetrazole (164 mg, 2.34 mmol) and dry dichloromethane (5 mL) was vigorously stirred at rt for 30 min under N_2, whereupon triol *48* (85 mg, 0.26 mmol) was added and stirring was continued for 30 min. The mixture was cooled to –78 °C and *m*CPBA (898 mg, 60%, 3.12 mmol) was added. The mixture was stirred at rt for 10 min, then was diluted with dichloromethane (50 mL). The solution was washed successively with 10% (w/v) aq. Na_2SO_3, sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate–hexane (8:2) as eluent to give the title compound as an oil (210 mg, 73%). $[\alpha]^{20}_D$ +9.4 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.40–7.13 (m, 35 H, ArC*H*), 5.16–4.87 (m, 13 H, H-1, 6 × $ArCH_2O$), 4.82–4.77 (m, 1 H, H-3'), 4.69–4.66 (m, 2 H, H-3, 0.5 × ArCH_2O), 4.49–4.40 (m, 2 H, H-4, 0.5 × ArCH_2O), 4.18–4.10 (m, 1 H, H-2'a), 4.03–3.92 (m, 2 H, H-5a, H-5b), 3.91–3.85 (m, 3 H, H-2'b, H-4', H-5'a), 3.74 (dd, 1 H, $J_{5'b,4'}$ 6.3 Hz, $J_{5'b5'a}$ 9.1 Hz, H-5'b) and 3.51 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 9.7 Hz, H-2); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.80 (C-1 of benzyl ether ring), 136.13–134.49 (6 × C-1 of benzyl ester rings), 132.95, 130.17, 128.72, 128.70, 128.63, 128.55, 128.43, 128.36, 128.29, 128.22, 128.16, 128.01, 127.83 (Ar), 96.90 (C-1), 78.53, 77.99, 76.32, 76.26, 75.92 (5 × CH), 74.14, 73.87 (CH_2) 70.79–70.79 (OCH_2Ar) and 60.33 (C-5); ^{31}P NMR ($CDCl_3$; 109 MHz)δ_p –0.55, –0.80 and –0.95; MS: (FAB) *m/z* 1129.3 [$(M+Na)^+$, 60%], 1107.3 [$(M+H)^+$, 80%], *m/z* calcd for $C_{58}H_{61}O_{16}P_3$ $(M+1)^+$ 1107.3250 found *m/z* 1107.3252:

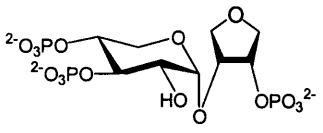
1-*O*-[(3'R,4'S)-3-hydroxytetrahydrofuran-4-yl] 2-*O*-benzyl-*β*-*D*-xylopyranoside 3,4,3'-tris(dibenzylphosphate) (55).



A mixture of bis(benzyloxy)(diisopropylamino) phosphine (260 mg, 0.75 mmol), 1*H*-tetrazole (79 mg, 1.13 mmol) and dry dichloromethane (5 mL) was vigorously stirred at rt for 30 min under N_2, whereupon triol **49** (40 mg, 0.13 mmol) was added and stirring was continued for 30 min. The mixture was cooled to –78 °C and *m*CPBA (260 mg, 60%, 1.5 mmol) was added. The mixture was stirred at rt for 10 min, then was diluted with dichloromethane (50 mL). The solution was washed successively with 10% (w/v) aq. Na_2SO_3, sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate–hexane (8:2) as eluent to give the title compound as an oil (107 mg, 79%).

$[\alpha]^{20}_D$ –10.8 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.37–7.04 (m, 35 H, ArC*H*), 5.02–4.71 (m, 14 H, H-3', 6.5 × ArCH_2O), 4.61–4.52 (m, 3 H, H-1, H-3, 0.5 × ArCH_2O), 4.37–4.29 (m, 1 H, H-4), 4.23 (dd, 1 H, $J_{2'a,3'a}$ 5.3, $J_{2'a,2'b}$ 10.2, H-2'a), 4.10 (dd, 1 H, $J_{5a,4}$ 5.3, $J_{5a,5b}$ 12.0, H-5a), 3.97–3.89 (m, 2 H, H-4', H-5'a), 3.82–3.78 (m, 2 H, H-2'b, H-5'b), 3.40–3.36 (m, 1 H, H-2) and 3.14 (dd, 1 H, $J_{5b,4}$ 9.4, $J_{5b,5a}$ 12.0, H-5b); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 138.11 (C-1 of benzyl ether ring), 136.13–135.71 (6 × C-1 of benzyl ester ring), 128.88, 128.82, 128.80, 128.75, 128.61, 128.56, 128.44, 128.41, 128.34, 128.32, 128.25, 128.22, 128.20, 128.05, 128.00, 127.89, 127.59 (ArCH), 102.86 (C-1), 79.20, 79.10 (2 × CH_2), 76.32, 76.26, 75.58, 75.54 and 74.29 (5 × CH), 73.79–69.43 (7 × ArCH_2O) and 62.89 (C-5); ^{31}P NMR ($CDCl_3$; 162 MHz) δ_p –0.25, –0.83 and –1.07; MS: (FAB) *m/z* 1129.4 $[(M+Na)^+$, 42%], 1107.4 $[(M+H)^+$, 80%], *m/z* calcd for $C_{58}H_{61}O_{16}P_3$ $[M+H]^+$ 1107.3250 found *m/z* 1107.3232:

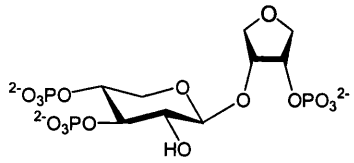
1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl] *α*-*D*-xylopyranoside 3,4,3'-trisphosphate (23).



10% Palladium on activated charcoal (200 mg) was added to a solution of compound **52** (94 mg, 0.084 mmol) in methanol (20 mL) and water (5 mL) and the mixture was shaken under an atmosphere of hydrogen at 50 psi at rt for 24 h. The suspension was filtered and washed well with de-ionised water. The combined filtrate was concentrated to a glassy clear solid. The residue was dissolved in de-ionised water (300 mL) and purified by ion exchange chromatography on Q Sepharose Fast flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of 8 eluted between 68% and 82%. Fractions containing the title compound, as judged by phosphate assay, were combined and evaporated to give a residue from which methanol (3 × 100 mL) was evaporated under reduced pressure to give the title trisphosphate as its triethylammonium salt (0.071 mmol, 85%).

$[\alpha]^{20}_{D}$ +30.2 (c 1.3, MeOH); ^{1}H NMR (D_2O; 400 MHz) δ_H 4.99 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.61–4.58 (m, 1 H, H-3'), 4.29–4.25 (m, 1 H, H-4'), 4.22–4.14 (m, 1 H, H-3), 3.97–3.91 (m, 1 H, H-4), 3.90–3.82 (m, 2 H, H-2'a, H-5'a), 3.75–3.67 (m, 3 H, H-2'b, H-5'b, H-5a), 3.58 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 8.8 Hz, H-2) and 3.52–3.45 (m, 1H, H-5b); ^{31}P NMR (D_2O; 162 MHz) δ_P 1.05 and 0.89 (2P); MS: (FAB) m/z 475.1 [$(M–H)^-$, 100 %], m/z calcd for $C_9H_{18}O_{16}P_3$ $[M–H]^-$ 474.9807 found m/z 474.9801:

1-*O*-[(3'S,4'R)-3-hydroxytetrahydrofuran-4-yl] *β*-*D*-xylopyranoside 3,4,3'-trisphosphate (24).

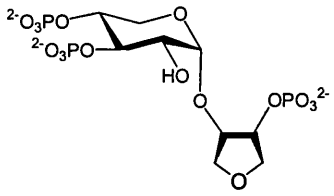


10% Palladium on activated charcoal (200 mg) was added to a solution of compound **53** (72 mg, 0.064 mmol) in methanol (20 mL) and water (5 mL) and the mixture was shaken under an atmosphere of hydrogen at 50 psi at rt for 24 h. The suspension was filtered and washed well with de-ionised water. The combined filtrate was concentrated to a glassy clear solid. The residue was dissolved in de-ionised water (300 mL) and purified by ion exchange chromatography on Q Sepharose Fast flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of 8 eluted between 68% and 82%. Fractions containing the title compound, as judged by phosphate assay, were combined and evaporated to give a

residue from which methanol (3 × 100 mL) was evaporated under reduced pressure to give the title trisphosphate as its triethylammonium salt (0.049 mmol, 77%).

$[\alpha]^{20}_D$ –17.7 (c 1.2, MeOH); ^{1}H NMR (CD_3OD; 400 MHz) δ_H 4.60–4.54 (m, 1 H, H-3'), 4.42–4.37 (m, 2 H, H-1, H-4'), 3.97–3.92 (m, 3 H, H-5a, H-5b, H-3), 3.88–3.83 (m, 2 H, H-2'a, H-5'a), 3.73–3.69 (m, 2 H, H-2'b, H-5'b), 3.38–3.34 (m, 1 H, H-2) and 3.31–3.23 (m, 1 H, H-4); ^{31}P NMR (CD_3OD; 162 MHz) δ_P 0.81, 1.05, 1.20; MS: (FAB) m/z 475.1 [$(M–H)^-$, 100 %], m/z calcd for $C_9H_{18}O_{16}P_3$ $[M–H]^-$ 474.9807 found m/z 474.9801:

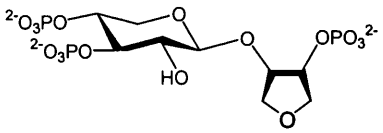
1-*O*-[(3'R,4'S)-3-hydroxytetrahydrofuran-4-yl] *α*-*D*-xylopyranoside 3,4,3'-trisphosphate (25).



10% Palladium on activated charcoal (200 mg) was added to a solution of compound **54** (110 mg, 0.099 mmol) in methanol (20 mL) and water (5 mL) and the mixture was shaken under an atmosphere of hydrogen at 50 psi at rt for 24 h. The suspension was filtered and washed well with de-ionised water. The combined filtrate was concentrated to a glassy clear solid. The residue was dissolved in de-ionised water (300 mL) and purified by ion exchange chromatography on Q Sepharose Fast flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of 8 eluted between 68% and 82%. Fractions containing the title compound, as judged by phosphate assay, were combined and evaporated to give a residue from which methanol (3 × 100 mL) was evaporated under reduced pressure to give the title trisphosphate as its triethylammonium salt (0.641 mmol, 65%).

$[\alpha]^{20}_D$ +40.8 (*c* 1.5, MeOH); ^{1}H NMR (D_2O; 270 MHz) δ_H 4.79 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.57–4.53 (m, 1 H, H-3'), 4.23–4.10 (m, 2 H, H-3, H-4'), 3.95–3.63 (m, 7 H, H-4, H-5a, H-5b, H-5'a, H-5'b, H-2'a, H-2'b) and 3.50 (dd, 1 H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.2 Hz, H-2); ^{31}P NMR (D_2O; 109 MHz) δ_p 0.34 and 0.80 (2P); MS: (FAB) *m/z* *m/z* 475.1 $[(M–H)^-$, 100 %], calcd for $C_9H_{18}O_{16}P_3$ $[M–H]^-$ 474.9807 found *m/z* 474.9802:

1-*O*-[(3'R,4'S)-3-hydroxytetrahydrofuran-4-yl] *β*-*D*-xylopyranoside 3,4,3'-trisphosphate (26).



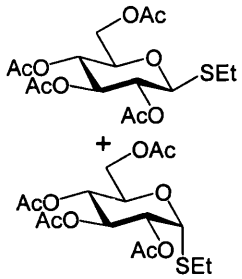
10% Palladium on activated charcoal (200 mg) was added to a solution of compound **55** (94 mg, 0.084 mmol) in methanol (20 mL) and water (5 mL) and the mixture was shaken under an atmosphere of hydrogen at 50 psi at rt for 24 h. The suspension was filtered and washed well with de-ionised water. The combined filtrate was concentrated to a glassy clear solid. The residue was dissolved in de-ionised water (300 mL) and purified by ion exchange chromatography on Q Sepharose Fast flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of 8 eluted between 68% and 82%. Fractions containing the title compound, as judged by phosphate assay, were combined and evaporated to give a residue from which methanol (3 × 100 mL) was evaporated under reduced pressure to give the title trisphosphate as its triethylammonium salt (0.071 mmol, 85%).

$[\alpha]^{20}_{D}$ –21.1 (*c* 0.52, MeOH); ^{1}H NMR (CD_3OD; 400 MHz) δ_H 4.81–4.78 (m, 1 H, H-3'), 4.54 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.45–4.40 (m, 1 H, H-4'), 4.30–4.19 (m, 1 H, H-2'a), 4.08–4.06 (m, 1 H, H-3), 3.98–3.92 (m, 1 H, H-5'a), 3.86 (dd, 1 H, $J_{2'b,3'}$ 3.5, $J_{2'b,2'a}$ 9.6, H-2'b), 3.73 (dd, 1 H, $J_{5'b,4'}$ 6.2, $J_{5'b,5'a}$ 8.8, H-5'b), 3.44–3.34 (m, 2 H, H-2, H-4) and 3.08–3.01 (m, 3 H, H-5a, H-5b, OH); ^{31}P NMR (CD_3OD; 162 MHz) δ_p 0.71, 1.32 and 1.47; MS: (FAB) *m/z* 950.9 [$(2M–H)^-$, 68%], 475.1 [$(M–H)^-$, 100 %], *m/z* calcd for $C_9H_{18}O_{16}P_3$ [$M–H]^-$ 474.9807 found *m/z* 474.9815:

7.5 Synthesis of adenophostin and analogues

7.5.1 Synthesis of disaccharide

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside (57a) and Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-glucopyranoside (57).



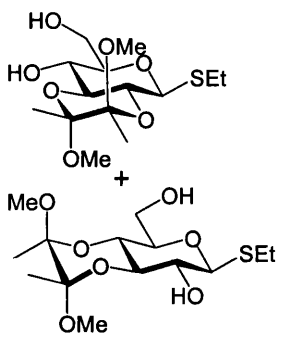
Sugar (10.0 g, 26 mmol) was dissolved in chloroform (200 mL) and cooled to 0 °C, ethanethiol (2.1 mL, 28 mmol) followed by $BF_3.(C_2H_5)_2O$ (16.3 mL, 128 mmol) was added slowly. The icebath was allowed to heat up and the mixture was stirred for 2 h. The reaction mixture was washed with H_2O (100 mL), satd aq $NaHCO_3$ (4 × 100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purified by flash chromatography on silica using diethyl ether–hexane (1:1) as eluent to give **57a** (150 mg, 1.5%), which was then crystallised from ethanol.

m.p. 94–96 °C; Lit m.p 91–93 °C[109]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.69 (d, 1 H, $J_{1,2}$ 5.9 Hz, H-1), 5.36 (t, 1 H, $J_{3,2}$=$J_{3,4}$ 9.8 Hz, H-3), 5.07–5.02 (m, 2 H, H-2, H–4), 4.46–4.42 (m, 1 H, H-5), 4.30 (dd, 1 H, $J_{6a,5}$ 4.7 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.08 (dd, 1 H, $J_{6b,5}$ 2.1 Hz, $J_{6b,6a}$ 12.5 Hz, H-6b), 2.63–2.50 (m, 2 H, SCH_2CH_3), 2.09 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO) and 1.27 (t, J 7.4 Hz, SCH_2CH_3):

Further elution gave **57** (9.5 g, 94%) which was crystallised from ethanol.

m.p.80–82 °C; Lit m.p 82–83 °C; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 5.23 (t, 1 H, $J_{3,4}$=$_{3,2}$ 9.3 Hz, H-3), 5.12-5.0 (m, 2 H, H-2, H-4), 4.50 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.25 (dd, 1 H, $J_{6a,5}$ 4.9 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.13 (dd, 1 H, $J_{6b,5}$ 2.4 Hz, $J_{6b,6a}$ 12.5 Hz, H-6b), 3.74–3.68 (m, 1 H, H-5), 2.78–2.64 (m, 2 H, SCH_2CH_3), 2.08 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 2.03 (s, 3 H, CH_3CO), 2.01 (s, 3 H, CH_3CO) and1.27 (t, 3 H, J 7.5 Hz, SCH_2CH_3):

(2'S,3'S) Ethyl 3,4-di-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-*β*-*D*-glucopyranoside (59).

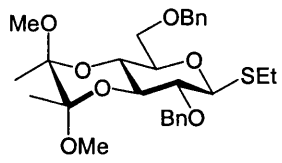


57 (5.0 g, 12.7 mmol) was deacetylated with NaOMe (35 mg, 0.64 mmol) in methanol (30 mL). When TLC (ethyl acetate) showed complete conversion of SM the reaction was neutralised with Dowex® 50WX4–50 ion-exchange resin and filtered. Trimethyl orthoformate, butane-2,3-dione and CSA were added and the mixture was heated until reflux. After 6 h, Et_3N was added and the mixture was concentrated under reduced pressure. It was purified by flash chromatography on silica using diethyl ether–hexane (1:1) as eluent which gave the title compound and its regioisomer as white foams. **59** (1.35 g, 31.3%)

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.29 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 3.75 (dd, 1 H, $J_{6a,5}$ 2.6 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 6.63–3.41 (m, 5 H, H-2, H-3, H-4, H-5, H-6b), 3.19 (s, 3H, OCH_3), 3.13 (s, 3 H, OCH_3), 2.65–2.59 (m, 2 H, SCH_2CH_3), 1.22 (s, 3 H, CH_3), 1.18 (s, 3 H, CH_3), 1.17 (t, 3 H, J 7.3 Hz, SCH_2CH_3):

Further elution gave 2,3 (1.6 g, 37%):

(2'S,3'S) Ethyl 2,6-di-*O*- benzyl 3,4-di-*O*-(2',3'-dimethoxybutane-2',3'-diyl)-1-thio-*β*-*D*-glucopyranoside (61).

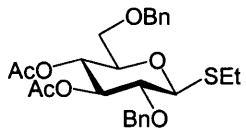


Sodium hydride (289 mg of an 60% w/w dispersion in mineral oil, 7.23 mmol) was added to a cooled solution (0 °C) of **59** (700 mg, 2.06 mmol) in DMF (10 mL) and benzyl bromide 0.49 mL, 4.55 mmol) was added slowly. It was allowed to warm up to RT and stirred for 3 h. The excess sodium hydride was destroyed with methanol (10 mL); and the reaction mixture was diluted with diethyl ether (100 mL) and H_2O (100 mL). The aqueous phase was extracted with diethyl ether and the organic extracts were washed with H_2O, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The compound was purified by crystallisation from hexane to yield the title compound (970 mg, 91%).

87–89 °C; Lit (white solid) ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.52–7.20 (m, 10 H, ArCH), 4.83 (AB, 2 H, J_{AB} 10.5 Hz, ArCH_2), 4.58 (AB, 2 H, J_{AB} 12.1 Hz, ArCH_2), 4.46 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 3.86 (t, 1 H, $J_{3,2}$=$J_{3,4}$ 9.4 Hz, H-3), 3.78–3.59 (m, 4 H, H-4, H-5, H-6a,

H-6b), 3.48 (t, 1 H, H-2), 3.29 (s, 3 H, OCH_3), 3.20 (s, 3 H, OCH_3), 2.81–2.65 (m, 2 H, SCH_2CH$_3$), 1.35 (s, 3 H, CH_3), 1.30 (t, 3 H, J 7.4 Hz, SCH$_2$CH_3) and 1.29 (s, 3 H, CH_3):

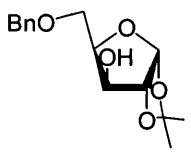
Ethyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-*β*-*D*-glucopyranoside (62)



61 (1.89 g, 3.64 mmol) was dissolved in 80% aq. AcOH (10 mL) and heated at reflux for 1 h. The mixture was concentrated under reduced pressure and co-evaporated with pyridine (3 × 10 mL). The mixture was dissolved in acetic anhydride (1.38 mL, 14 mmol) and pyridine (5 mL) and stirred overnight. The mixture was concentrated under reduced pressure from toluene (3 × 10 mL). The product was isolated as white needles after crystallisation from diethyl ether–hexane (5:2, v/v), (1.3 g, 73%).

m.p. 108 °C; Lit m.p 108 °C[71]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.52–7.27 (m, 10 H, ArH), 5.18 (t, 1 H, $J_{3,2=3,4}$ 9.4 Hz, H-3), 4.99 (t, 1 H, H-4), 4.86 (AB, 1 H, J_{AB} 10.9 Hz, 0.5 × OCH_2Ar), 4.58–4.47 (m, 4 H, $J_{1,2}$ 9.8 Hz, H-1, 1.5 × OCH_2Ar), 3.66–3.45 (m, 4 H, H-2, H-5, H-6a, H-6b), 2.84–2.71 (m, 2 H, SCH_2CH$_3$), 1.90 (s, 3 H, CH_3CO), 1.89 (s, 3 H, CH_3CO),and 1.33 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH_3); MS: (FAB) *m/z* calcd for $C_{26}H_{32}O_7S$ $[M+H]^+$ 488.1868 found *m/z* 488.1838:

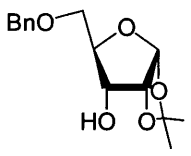
5-*O*-Benzyl-1,2-*O*-isopropylidene-*α*-*D*-xylofuranose (63).



A mixture of 1,2-*O*-isopropylidene-*α*-D-xylofuranose (5.64 g, 29.7 mmol), dibutyltinoxide (8.1 g, 32.6 mmol), tetrabutylammoniumbromide (9.5 g, 29.7 mmol), benzyl bromide (5.3 mL, 44.5 mmol) and acetonitrile (300 mL) was heated under reflux for 24 h via a soxhlet thimble containing 3 Å molecular sieves. After this time the milky suspension had become a pale yellow solution. The solution was cooled, Et_3N (50 mL) was added and the mixture was heated under reflux for a further 1 h. The mixture was cooled, diethyl ether (200 mL) and satd aq. $NaHCO_3$ (200 mL) were added and stirred vigorously for 1 h followed by filtration through celite to remove the tin residues. The diethyl ether was separated and dried ($MgSO_4$), filtered and concentrated under reduce pressure. It was purified by flash chromatography on silica using ethyl acetate–hexane (1:1) as eluent to give a white solid which was crystallised from hexane to give the title compound. (7.26 g, 87%).

m.p.64 °C; Lit m.p 60–62 °C [110]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.39–7.28 (m, 5 H, ArC*H*), 5.96 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.63, 4.57 (AB, 2 H, J_{AB} 12.1, OCH_2Ar), 4.49 (d, 1 H, $J_{2,1}$ 3.7 Hz, H-2), 4.29–4.23 (m, 2 H, H-3, H-4), 3.95–3.88 (m, 2 H, H-5a, H-5b), 3.66 (br s, 1 H, OH-3), 1.47 (s, 3 H, isopropylidene CH_3) and 1.31 (s, 3 H, isopropylidene CH_3):

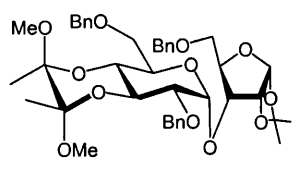
5-*O*-Benzyl-1,2-*O*-isopropylidene-*α*-*D*-ribofuranose (64).



A solution of **63** (5 g, 17.8 mmol) in dry DMSO (20 mL) was added dropwise to a solution of acetic anhydride (40 mL) in dry DMSO (30 mL) and the solution was stirred under N_2 at 23 °C overnight. The reaction mixture was then added to a vigourously stirred solution of satd aq. $NaHCO_3$ (300 mL) at 0 °C and stirring was continued for 1 h. The mixture was extracted with dichloromethane and the combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The yellow oil was dissolved in ethanol/H_2O (1:1, 200 mL) and $NaBH_4$ (742 mg, 19.6 mmol) was added in portions and stirring was continued for 1 h. The mixture was partially evaporated and the aq. layer was extracted with dichloromethane, dried ($MgSO_4$), filtered and concentrated under reduced pressure. It was purified by flash chromatography on silica using chloroform as eluent to give the title compound which was crystallised from hexane (2.52 g, 50.5%).

m.p.80–82 °C; Lit m.p 81–83°C[111]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.35–7.29 (m, 5 H, ArC*H*), 5.81 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.60 (s, 2 H, OCH_2Ar), 4.56 (t, 1 H, H-2), 3.96–3.89 (m, 2 H, H-3, H-4), 3.79 (dd, 1 H, $J_{5a,4}$ 10.9 Hz, $J_{5a,5b}$ 2.7 Hz, H-5a), 3.64 (dd, 1 H, $J_{5b,4}$ 4.7 Hz, H-5b), 2.33 (d, 1 H, J 9.7 Hz, OH-3), 1.56 (s, 3 H, isopropylidene CH_3) and 1.37 (s, 3 H, isopropylidene CH_3):

(2"S,3"S) 5-*O*-Benzyl-3-O-[2',6'-di-O-benzyl-3',4'-di-*O*-(2",3"-dimethoxy-butane-2",3"-diyl)*α*-*D*-glucopyranosyl]-1,2-*O*-isopropylidene-*β*-*D*-ribofuranoside (67)

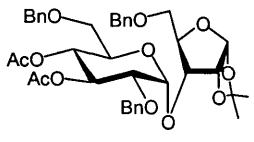


Acceptor (**64**) (350 mg, 1.25 mmol) and donor (**61**) (973 mg, 1.87 mmol) were dissolved in anhydrous diethyl ether (20 mL). Activated 4Å molecular seives were added and the mixture was stirred under argon. NIS (476 mg, 2.11 mmol) and a catalytic amount of TfOH (10 μL) were added. After stirring for 15 min., TLC (diethyl ether–hexane 2:1) showed complete disappearence of acceptor. The reaction mixture was

filtered though celite and the filtrate was diluted with ethyl acetate, washed with satd aq. $Na_2S_2O_3$ (15 mL, 1 M) and satd aq. $NaHCO_3$ (15 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. It was purified by flash chromatography on silica using diethyl ether–hexane (2:3) as eluent to yield the title compound as an oil (560 mg, 61%).

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.22–7.41 (m, 15 H, ArC*H*), 5.79 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 5.19 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1'), 4.78, 4.75 (AB, 2 H, J_{AB} 12.1 Hz, OCH_2Ar), 4.70 (t, 1 H, $J_{2,3}$=$J_{2,1}$ 3.9 Hz, H-2), 4.60 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.52 (AB, 2 H, J_{AB} 11.7 Hz, OCH_2Ar), 4.46 (AB, 1 H, 0.5 × OCH_2Ar), 4.31 (dd, 1 H, $J_{3,2}$ 3.9 Hz, $J_{3,4}$ 9.0 Hz, H-3), 4.13–4.08 (m, 2 H, H-3', H-4'), 3.84–3.54 (m, 7 H, H-4, H-5a, H-5b, H-2', H-5', H-6'a, H-6'b), 3.30 (s, 3H, OCH_3), 3.18 (s, 3 H, OCH_3), 1.53 (s, 3 H, isopropylidene CH_3), 1.34 (s, 6 H, BDA CH_3 and isopropylidene CH_3) and 1.30 (s, 3 H, BDA CH_3); ^{13}C NMR (100 MHz; $CDCl_3$) 139.12, 138.34, 138.17 (C-1 of benzyl ether ring), 128.52, 128.43, 128.30, 127.80, 127.71, 127.66, 127.48 (Ar*C*H), 113.14 (isopropylidene *C*$(CH_3)_2$), 104.43 (C-1), 99.90, 99.73 (BDA O*C*CH_3OCH_3), 96.10 (C-1'), 78.17, 76.92, 76.45 (3 × CH), 73.82 (O*C*H_2Ar), 73.79 (CH), 72.35 (O*C*H_2Ar), 69.82, 69.65 (2 × CH), 68.56, 68.12 (C-6, C-5'), 48.51, 48.32 (2 × O*C*H_3), 27.18, 27.07 (2 × isopropylidene CH_3) and 18.39, 18.17 (2 × BDA *C*H_3). MS: (FAB) 759.4 $[(M+Na)^+$ 78%), *m/z* calcd for $C_{41}H_{52}O_{12}$ $[M+Na]^+$ 759.3356 found *m/z* 759.3324:

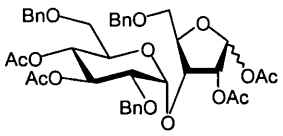
3',4'-Di-*O*-acetyl-2',5,6'-tri-*O*-benzyl-3-*O*-α-*D*-glucopyranosyl-1,2-*O*-isopropylidene-α-*D*-ribofuranose (65).



Acceptor (**64**) (478 mg, 1.7 mmol) and donor (**62**) (1.0 g, 2.05 mmol) were dissolved in a mixture of toluene (8 mL) and 1,4-dioxane (24 mL). Activated 4Å molecular seives were added and the mixture was stirred under argon. NIS (518 mg, 2.3mmol) and a catalytic amount of TfOH (15 μL) were added. After stirring for 15 min., TLC (diethyl ether–hexane 2:1) showed complete disappearence of acceptor. The reaction mixture was filtered though celite and the filtrate was diluted with ethyl acetate, washed with aq. $Na_2S_2O_3$ (15 mL, 1 M) and satd. aq. $NaHCO_3$ (15 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. It was purified by flash chromatography on silica using diethyl ether–hexane (1:1) as eluent to give the title compound (810 mg, 68%) which was recrystallised from dissopropylether.

m.p.124–126 °C; Lit m.p 125–127 °C[64]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.33–7.23 (m, 15 H, ArCH), 5.82 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.37 (dd, 1 H, $J_{3',4'}$=$J_{3',2'}$ 9.8 Hz, H-3'), 5.21 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1'), 5.09 (dd, 1 H, $J_{4',3'}$ 9.8 Hz, H-4'), 4.72–4.48 (m, 6 H, 2.5 × OCH_2Ar, H-2), 4.32–4.29 (m, 2 H, H-4, 0.5 × OCH_2Ar), 4.17 (dd, 1 H, $J_{3,2}$ 4.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 3.83–3.79 (m, 2 H, H-5a, H-5'), 3.71 (dd, 1 H, $J_{5b,4}$ 3.5 Hz, $J_{5b,5a}$ 11.7, H-5b), 3.60 (dd, 1 H, $J_{2',3'}$=$J_{2',1'}$ 3.5 Hz, H-2'), 3.34 (dd, 1H, $J_{6a,5}$ 2.4 Hz, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.29 (dd, 1 H, $J_{6b,5}$ 3.5 Hz, $J_{6b,6a}$ 10.9 Hz, H-6b), 2.03 (s, 3 H, CH_3CO), 1.88 (s, 3 H, CH_3CO), 1.53 (s, 3 H, isopropylidene CH_3) and 1.36 (s, 3 H, isopropylidene CH_3):

1,2,3',4'-Tetra-*O*-acetyl-2',5,6'-O-benzyl-3-*O*-*α*-*D*-glucopyranosyl-*D*-ribofuranose (56).



Method A (from 65)

65 (750 mg, 1.06 mmol) Was treated with 90% aq. TFA (5 mL) and stirred for 10 min. The reaction was quenched by adding satd. aq. $NaHCO_3$ (100 mL) and extracted with ethyl acetate(200 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. It was stirred in a mixture of acetic anhydride (1 mL) and pyridine (7.5 mL) overnight and concentrated under reduced pressure. Purification by flash chromatography on silica using ethyl acetate–hexane (3:7) as eluent to give the title compound (700 mg, 88%) which was crystallised from ethyl acetate–hexane (1:4):

Method B (from 67)

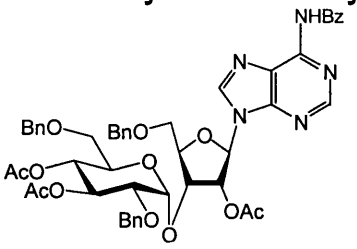
The **67** (1.4 g, 1.89 mmol) was heated atreflux in a mixture of acetic anhydride–H_2O–ethylene glycol (14:6:3) for 75 min. It was quenched with satd aq. $NaHCO_3$ (100 mL) and extracted with ethyl acetate (3 × 100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was dissolved in a mixture of acetic anhydride (5 mL) and pyridine (10 mL) and stirred overnight. The mixture was diluted with toluene and concentrated under reduced pressure. It was purified by flash chromatography on silica using ethyl actetate–hexane (3:7) as eluent to yield the title compound (600 mg, 42%) which was crystallised from ethyl acetate–hexane (1:4).

m.p.104–106 °C; Lit m.p 105–107 °C[64]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.32–7.22 (m, 15 H, ArC*H*), 6.12 (s, 1 H, H-1), 5.37 (t, 1 H, $J_{3',2'}$ 9.5 Hz, H-3'), 5.33 (d, 1 H, $J_{2,1}$ 4.8 Hz, H-2), 5.08–5.03 (m, 2 H, H-1', H-4'), 4.66–4.62 (m, 2 H, H-3, 0.5 × OCH_2Ar), 4.56–4.47 (m, 4 H, 2 × OCH_2Ar) 4.39–4.36 (m, 1 H, H-4), 4.29 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 ×

OCH_2Ar), 3.87 (ddd, 1 H, J 3.1 Hz, J 10.2 Hz, J 6 Hz, H-5'), 3.72 (dd, 1 H, $J_{5a,4}$ 2.7 Hz, $J_{5a,5b}$ 11.3 Hz, H-5a), 3.63 (dd, 1 H, $J_{5b,4}$ 3.5 Hz, $J_{5b,5a}$ 11.3, H-5b), 3.56 (dd, 1 H, $J_{2',3'}$ 10.1 Hz, $J_{2',1'}$ 3.5 Hz, H-2'), 3.35 (dd, 1 H, $J_{6'a,5'}$ 2.3 Hz, $J_{6'a,6'b}$ 10.9 Hz, H-6'a), 3.29 (dd, 1 H, $J_{6'b,5'}$ 3.9 Hz, $J_{6'b,6'a}$ 10.9 Hz, H-6'b), 1.95 (s, 3 H, CH_3CO), 1.93 (s, 3 H, CH_3CO), 1.87 (s, 3 H, CH_3CO) and 1.86 (s, 3 H, CH_3CO):

7.5.2 Synthesis of adenophostin A

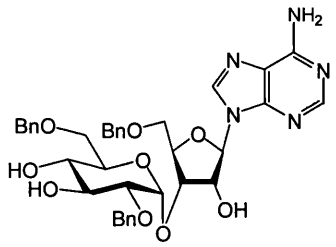
2'-*O*-Acetyl-3'-*O*-(3,4-di-*O*-acetyl-6-*O*-benzyl-α-*D*-glucopyranosyl)-6-N-benzoyl-5'-*O*-benzyladenosine (68).



A suspension of *N*-6-benzoyl-adenine (430 mg, 1.79 mmol) in HMDS (4.5 mL) and pyridine (1.6 mL) was heated at reflux overnight. The reaction mixture was cooled, diluted with toluene (5 mL) and concentrated. The residue was repeatedly diluted with toluene (3 × 5 mL) and concentrated under reduced pressure to remove excess HMDS. A mixture of **56** (500 mg, 0.67 mmol) in dichloroethane (10 mL) and a catalytic amount of TMSOTf (40 μl) was added to the silylated N-6-benzoyladenine. After stirring at reflux for 7 h, TLC (toluene–ethyl acetate–methanol 36:10:1) showed conversion of the dissacharide into a lower-running product. The reaction was quenched by adding Et_3N (1 mL), diluted with dichloromethane (20 mL) and poured into satd aq. $NaHCO_3$ (10 mL). The organic phase was washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. It was purified by flash chromatography on silica using dichloromethane–methanol (99:1) as eluent to give the title compound (630 mg, 88%).

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 9.05 (s, 1 H, NH),8.80 (s, 1 H, H-2), 8.38 (s, 1 H, H-8), 8.6–8.04 (m, 2 H, H-2 and H-6 of Bz ring), 7.64–7.60 (m, 1 H, H-4 of Bz ring), 7.55–7.52 (m, 2 H, H-3 and H-5 of Bz ring), 7.47–7.21 (m, 15 H, ArCH), 6.42 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1'), 5.73 (t, 1 H, $J_{2',1'=2',3'}$ 5.5 Hz, H-2'), 5.44 (t, 1 H, $J_{3'',4''=3'',2''}$ 9.8 Hz, H-3''), 5.04 (t, 1 H, $J_{4'',3''=4'',5''}$ 9.8 Hz, H-4''), 4.97 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.74 (dd, 1 H, $J_{3',4'}$ 3.9 Hz, $J_{3',2'}$ 5.5 Hz, H-3'), 4.69, 4.36 (OCH_2Ar), 4.61–4.43 (m, 5 H, H-4', 2 × OCH_2Ar), 4.01–3.97 (m, 1 H, H-5''), 3.73 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.5 Hz, H-5a'), 3.62 (dd, 1 H, $J_{5'b,4'}$ 2.7 Hz, $J_{5'b,5'a}$ 10.5 Hz, H-5'b), 3.56 (dd, 1 H, $J_{2'',1''}$ 3.5 Hz, $J_{2'',3''}$ 9.8 Hz, H-2''), 3.43–3.37 (m, 2 H, H-6''a, H-6''b), 1.99 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), and 1.88 (s, 3H, CH_3CO):

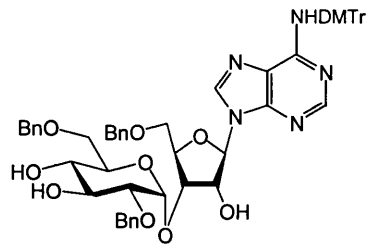
2",5',6"-Tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl adenosine (**69**).



Methanol (2 mL) was added to a solution of LiOH (158 mg, 3.7 mmol) in water (1 mL). **68** (350 mg, 0.37 mL) was dissolved in THF(2 mL). The LiOH solution was added and the mixture was stirred overnight. The mixture was diluted with water (40 mL) and the THF/methanol was removed by concentrating under reduced pressure. It was extracted with chloroform (3 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to leave an oil. This was purified by flash chromatography on silica using ethyl acetate–ethanol (14:1) as eluent to give to give the title compound as a colourless oil (180 mg, 68%) which was crystallised from methanol.

m.p.95–98 °C; lit white solid[60]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.29 (s, 1 H, H-8), 8.06 (s, 1 H, H-2), 7.49–7.23 (m, 15 H, ArC*H*), 6.19 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.86 (br s, 2 H, NH_2), 4.85 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.71 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.63–4.44 (m, 9 H, H-2', 2.5 × OCH_2Ar, 3 × OH), 4.40–4.25 (m, 1 H, H-4'), 4.13 (t, H-3'), 3.94–3.90 (m, 1 H, H-3") 3.73–3.65 (m, 4 H, H-4", H-5", H-6"a, H-6"b), 3.60 (dd, 1 H, $J_{5'a,4'}$ 2.5 Hz, $J_{5'a,5'b}$ 10.5 Hz, H-5'a), 3.54 (dd, 1 H, $J_{5'b,4'}$ 2.7 Hz, $J_{5'b,\ 5'a}$ 10.5 Hz, H-5'b) and 3.43 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 10.3 Hz, H-2"):

2",5',6"-Tri-O-benzyl-3'-*O*-α-*D*-glucopyranosyl)-N-6-dimethoxytrityl-9-β-*D*-ribofuranosidopurine (70).

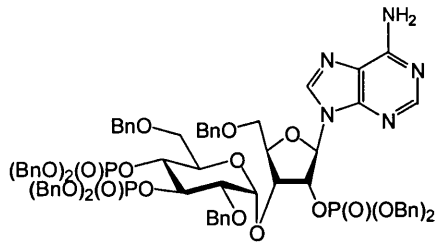


To a solution of **69** (150 mg, 0.21 mmol) in dry pyridine (5 mL) was added DMTrCl (267 mg, 0.75 mmol) and DMAP (50 mg) and the mixture was stirred for 48 h. The pyridine was removed under reduced pressure and coevaporated with toluene to give an oil. The residue was purified by flash chromatography on silica using ethyl acetate–hexane–triethylamine (1:1:0.1 then 1:0:0.1) as eluent to give the title compound (150 mg, 70%) as a yellow oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.04 (s, 1 H, H-8), 7.92 (s, 1 H, H-2), 7.36–7.15 (m, 23 H, ArC*H*), 6.98–6.78 (m, 5 H, ArC*H*), 6.25 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1'), 4.71 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.58 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.53–4.38 (m, 6 H, H-2', H-

4', 2 × OCH_2Ar), 4.37 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.21–4.19 (m, 2 H, H-3', H-3"), 3.97–3.93 (m, 1 H, H-5"), 3.78 (s, 6H, 2 × OCH_3 of DMTr), 3.78–3.58 (m, 3 H, H-4", H-6"a, H-6"b), 3.49–3.51 (m, 2 H, H-5'a, H-5'b) and 3.37 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.8 Hz, H-2"); MS: (FAB) *m/z* calcd for $C_{58}H_{59}N_5O_{11}$ $[M+H]^+$ 1003.4322 found *m/z* 1003.4338:

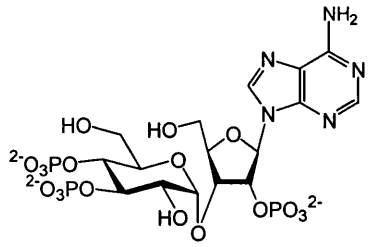
2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl adenosine (71).



Bis(benzyloxy)(diisopropylamino)phosphine (164 mg, 0.48 mmol) and 1*H*-tetrazole (50 mg, 0.71 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to the triol (**70**) (80 mg, 0.079 mmol). After a further 20 min, TLC (ethyl acetate–hexane 8:2) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (161 mg, 0.55 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and dichloromethane (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was dissolved in acetic acid (80%, 5 mL) and stirred for 40 min. After this time it was diluted with water (20 mL) and concentrated under reduced pressure to leave an oil which was purified by flash chromatography on silica using chloroform–acetone (4:1)as eluent to give the title compound (100 mg, 85%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.24 (s, 1 H, H-8), 7.89 (s, 1 H, H-2), 7.39–6.95 (m, 45 H, ArC*H*), 6.34 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.64–5.58 (m, 3 H, H-2', NH_2), 5.32 (d, 1 H, $J_{1",2"}$ 3.3 Hz, H-1"), 5.04–4.86 (m, 8 H, 3.5 × OCH_2Ar, H-3"), 4.79–4.36 (m, 13 H, 5 × OCH_2Ar, H-3', H-4', H-4"), 4.29 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 3.85–3.79 (m, 1 H, H-5") and 3.67–3.53 (m, 5 H, H-5'a, H-2", H-5'b, H-6"a, H-6"b); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P –0.15, –0.83, –1.01:

3-*O*-α-*D*-Glucopyranosyl adenosine 2',3",4"-trisphosphate (72)

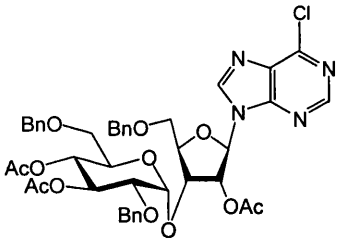


A mixture of **71** (60 mg, 0.004 mmol) and $Pd(OH)_2$ on carbon (10%, 120 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 65 °C for 3 h. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure to give the title trisphosphate (22 mg, 81%) as its sodium salt.

^{1}H NMR (400 MHz; D_2O) δ_H 8.15 (s, 1 H, H-8), 8.04 (s, 1 H, H-2), 6.11 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.19 (d, 1 H, $J_{1",2"}$ 3.9 Hz, H-1"), 5.15–5.10 (m, 1 H, H-2'), 4.49–4.47 (1 H, m, H-3'), 4.36–4.26 (m, 2 H, H-4', H-3"), 3.89–3.82 (m, 1 H, H-4") and 3.77-3.58 (m, 6 H, H-5'a, H-5'b, H-2", H-5", H-6"a, H-6"b); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 2.84, 2.27 and 1.09:

7.5.3 Synthesis of OMe adenophostin

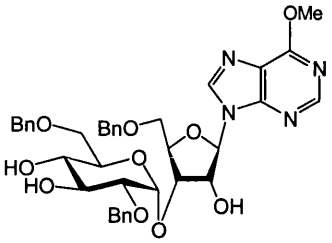
2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-chloro-9-β-*D*-ribofuranosylpurine (73).



TMSOTf (0.48 mL, 2.65 mmol) was added cautiously dropwise to a stirred solution of **56** (500 mg, 0.66 mmol), 6-chloropurine (113 mg, 0.73 mmol) and DBU (0.3 mL, 2.00 mmol) in acetonitrile (5 mL) at 0 °C. The mixture was heated at 60 °C for 1 h, after which it was cooled and quenched by careful addition of sat. aq. $NaHCO_3$ solution (25 mL). The mixture was extracted with dichloromethane (3 × 30 mL) and the combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave a yellow oil. Purification by flash chromatography on silica using dichloromethane–acetone (40:1) as eluent gave the title compound (500 mg, 89%) as a colourless oil.

$[\alpha]^{20}_D$ +71.0 (*c* 1.14,$CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.73 (s, 1 H, H-8), 8.48 (s, 1 H, H-2), 7.37–7.23 (m, 15 H, ArC*H*), 6.41 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1'), 5.69 (t, 1 H, $J_{2',1'} = {}_{2',3'}$ 5.5 Hz, H-2'), 5.44 (t, 1 H, $J_{3",4"} = J_{3",2"}$ 9.8 Hz, H-3"), 5.02 (t, 1 H, $J_{4",3"} = J_{4",5"}$ 9.8 Hz, H-4"), 4.96 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.71 (dd, 1 H, $J_{3',4'}$ 3.9 Hz, $J_{3',2'}$ 5.3 Hz, H-3'), 4.62 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.56–4.48 (m, 5 H, 2 × OCH_2Ar, H-4'), 4.34 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.00–3.96 (m, 1 H, H-5"), 3.70 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5'a), 3.60 (dd, 1 H, $J_{5'b,4'}$ 2.7 Hz, $J_{5'b,5'a}$ 10.9 Hz, H-5'b), 3.56 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.8 Hz, H-2"), 3.40–3.36 (m, 2 H, H-6"a, H-6"b), 1.99 (s, 3 H, CH_3CO), 1.94 (s, 3 H, CH_3CO) and 1.87 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz; $CDCl_3$) 169.93, 169.86, 169.42 (3 × O*C*OCH_3), 151.90 (C-4 or C-6), 151.23 (C-2), 150.86 (C-4 or C-6), 143.49 (C-8), 137.29, 137.14, 136.75 (3 × C-1 Bn ether ring), 131.77 (C-5), 128.45, 128.28, 128.16, 127.94, 127.75, 127.64, 127.58 (Ar*C*H), 97.97 (C-1"), 86.51 (C-1'), 83.03 (CH), 77.00 (CH), 76.69 (CH), 76.57 (CH), 74.59 (CH) , 76.63, 73.45, 73.22 (3 × OCH_2Ar), 71.65 (CH), 69.22, 67.89 (C-5', C-6"), 20.91, 20.72, 20.37 (3 × CH_3*C*O); MS: (FAB) *m/z* 845.3 [(M), 65%], *m/z* calcd for $C_{43}H_{44}N_4O_{12}Cl$ $[M+H]^+$ ^{37}Cl, 847.2771 found *m/z* 847.2738, $[M+H]^+$ ^{35}Cl, 846.2834 found *m/z* 846.2820; Anal. Calcd for $C_{43}H_{44}N_4O_{12}$ Cl C, 61.19; H, 5.43; N 6.64%. Found: C, 61.60; H, 5.43; N 6.55%:

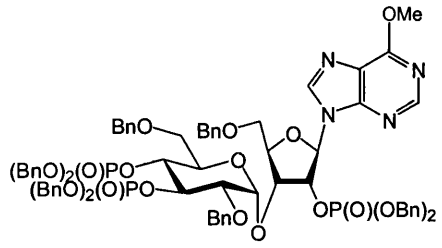
2",5',6"-Tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-methoxy-9-β-*D*-ribofuranosylpurine (74).



A solution of 1 M NaOMe (0.8 mL, 0.8 mmol) was added to a stirred solution of **73** (400 mg, 0.4 mmol) in methanol. The mixture was stirred for 30 min after which it was neutralised with Dowex® 50WX4–50 ion-exchange resin and filtered. The filtrate was concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (4:1) as eluent to give the title compound as a colourless oil (250 mg, 74%).

$[\alpha]^{20}_{D}$ +16.7 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.44 (s, 1 H, H-8), 8.22 (s, 1 H, H-2), 7.37–7.24 (m, 15 H, ArC*H*), 6.27 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 4.79 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.69 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.62–4.56 (m, 1 H, H-2'), 4.53–4.41 (m, 6 H, 2.5 × OCH_2Ar, H-4'), 4.21–4.19 (m, 4 H, H-3', OCH_3), 4.10 (t, 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.4 Hz, H-3"), 3.96–3.91 (m, 1 H, H-5"), 3.71–3.65 (m, 3 H, H-4", H-6"a, H-6"b), 3.60 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.5 Hz, H-5'a), 3.54 (dd, 1 H, $J_{5'b,4'}$ 2.7 Hz, $J_{5'b,\ 5'a}$ 10.5 Hz, H-5'b) and 3.43 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.4 Hz, H-2"); ^{13}C NMR (100 MHz; $CDCl_3$) 160.93 (C-4 or C-6), 151.71 (C-2), 151.49 (C-4 or C-6), 140.48 (C-8), 137.62, 137.06, 136.51 (3 × C-1 Bn ether ring), 128.61, 128.56, 128.46, 128.39, 128.23, 127.91, 127.70, 127.61, 127.53 (Ar*C*H), 121.68 (C-5), 99.60 (C-1"), 88.02 (C-1'), 83.07 (CH), 80.56 (CH), 79.04 (CH), 76.05 (CH), 74.02 (CH), 71.82 (CH), 73.69, 73.60, 72.77 (3 × O*C*H_2Ar), 69.92 (CH), 69.81, 69.34 (C-5', C-6") and 54.33 (OCH_3); MS: (FAB) *m/z* 715.3 [$(M+H)^+$, 11%], *m/z* calcd for $C_{38}H_{42}N_4O_{10}$ $[M+H]^+$, 715.2979 found *m/z* 715.2983:

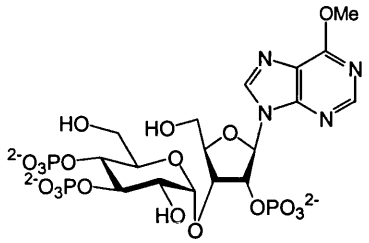
2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 6-methoxy-9-β-*D*- ribofuranosylpurine (75).



Bis(benzyloxy)(diisopropylamino)phosphine (405 mg, 1.17 mmol) and 1*H*-tetrazole (123 mg, 1.76 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **74**. After a further 20 min, TLC (ethyl acetate:methanol, 95:5) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (360 mg, 1.25 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and ethyl acetate (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate-hexane (1:1 then 4:1) to give the title compound (200 mg, 69%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.40 (s, 1 H, H-8), 8.07 (s, 1 H, H-2), 7.40–6.94 (m, 45 H, ArC*H*), 6.40 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H-1'), 5.62–5.57 (m, 1 H, H-2'), 5.33 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 5.06–4.87 (m, 8 H, 3.5 × OCH_2Ar, H-3"), 4.80–4.35 (m, 13 H, 5 × OCH_2Ar, H-3', H-4', H-4"), 4.30 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.14 (s, 3 H, OCH_3), 3.86–3.83 (m, 1 H, H-5"), 3.71–3.57 (m, 4 H, H-5'a, H-5'b, H-6"a, H-6"b) and 3.43 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.7 Hz, H-2"); ^{13}C NMR (100 MHz; $CDCl_3$) 161.03 (C-4 or C-6), 152.31 (C-2), 152.11 (C-4 or C-6), 138.20 (C-8), 137.84, 137.43, 136.40 (3 × C-1 Bn ether ring), 136.33–135.09 (C-1 of bezylphosphoro ring), 128.73, 128.69, 128.65, 128.54, 128.51, 128.29, 128.27, 128.10, 128.06, 128.00, 127.94, 127.91, 127.86, 127.80 (ArCH), 121.98 (C-5), 95.68 (C-1"), 85.87 (C-1'), 82.84 (CH), 78.34 (CH), 77.77 (CH with C-P couplings), 74.70, 73.97, 73.70 (3 × OCH_2Ar), 71.92 (CH with C-P coupling), 70.45 (CH with C-P coupling), 70.19 (C-5' or C-6"), 70.13–69.49 (POCH_2Ar with C-P coupling), 68.71 (C-5' or C-6") and 54.52 (OCH_3); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P –0.37, –0.97, –1.14; MS: (FAB) *m/z* 1496.0 [$(M+H)^+$, 78%], *m/z* calcd for $C_{80}H_{81}N_4O_{19}P_3$ $[M+H]^+$, 1495.4786 found *m/z* 1495.4815:

3'-*O*-α-*D*-Glucopyranosyl-6-methoxy-9-β-D-ribofuranopurine 2',3",4"-trisphosphate (76).

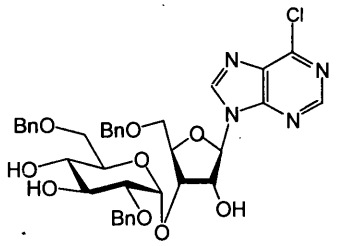


A mixture of **75** (35 mg, 0.023 mmol) and $Pd(OH)_2$ on carbon (10%, 110 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 65 °C for 31 h. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated under reduced pressure to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure to give the title trisphosphate (0.019 mmol, 83%) as its sodium salt.

^{1}H NMR (400 MHz; D_2O) δ_H 8.32 (s, 1 H, H-8), 8.28 (s, 1 H, H-2), 6.14 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H-1'), 5.19 (d, 1 H, $J_{1",2"}$ 3.9 Hz, H-1"), 5.10–5.04 (m, 1 H, H-2'), 4.55–4.48 (1 H, m, H-3'), 4.38–4.23 (m, 2 H, H-4', H-4"), 3.97 (s, 3 H, OCH_3) and 3.83-3.52 (m, 7 H, H-5'a, H-5'b, H-2", H-3", H-5", H-6"a, H-6"b); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 5.64, 4.93 and 4.87; MS: (FAB) *m/z* 684.1 [$(M-H)^-$, 28%], *m/z* calcd for $C_{17}H_{27}N_4O_{19}P_3$ [M–H]$^-$, 684.0437 found *m/z* 684.0447:

7.5.4 Synthesis of 6-cyclopentylamino adenophostin

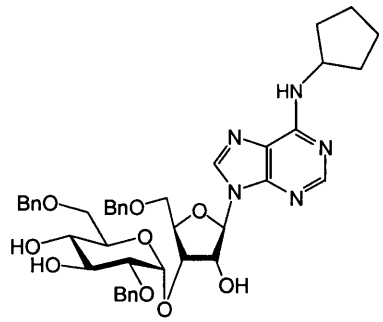
2",5',6"-Tri-O-benzyl-3'-O-α-D-glucopyranosyl-6-chloro -9-β-D-ribofuranosylpurine (74).



A catalytic amount of NaOMe (30 mg, mmol) was added to a stirred solution of **73** (300 mg, 0.35 mmol) in methanol. The mixture was stirred for 1.5 h after which it was neutralised with Dowex® 50WX4–50 ion-exchange resin and filtered. The filtrate was concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (7:3) as eluent to give the title compound as a colourless oil (140 mg, 55%).

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.65 (s, 1 H, H-8), 8.44 (s, 1 H, H-2), 7.37–7.23 (m, 15 H, ArC*H*), 6.23 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H-1'), 4.81 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.70 (AB, 1 H, 0.5 × OCH_2Ar), 4.68–4.64 (m, 1 H, H-2'), 4.56–4.40 (m, 7 H, 2.5 × OCH_2Ar, H-2', H-4', O*H*), 4.26 (br s, 1 H, O*H*), 4.22 (dd, 1 H, $J_{3'4'}$ 2.7 Hz, $J_{3',2'}$ 5.5 Hz, H-3'), 4.06 (t covered by s, $J_{3",2"}$=$J_{3",4"}$ 9.7 Hz, H-3", O*H*), 3.92–3.88 (m, 1 H, H-5"), 3.68–3.65 (m, 3 H, H-4", H-6"a, H-6"b), 3.62 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5'a), 3.55 (dd, 1 H, $J_{5'b,4'}$ 2.3 Hz, $J_{5'b,5'a}$ 10.9 Hz, H-5'b) and 3.43 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.7 Hz, H-2"); MS: (FAB) *m/z* 719.1 [(M+H)$^+$, 32%], *m/z* calcd for $C_{37}H_{39}N_4O_9Cl$ [M+H]$^+$ ^{37}Cl, 721.2454 found *m/z* 721.2481, [M+H]$^+$ ^{35}Cl, 719.2483 found *m/z* 719.2478:

2",5',6"-Tri-O-benzyl-3'-O-α-D-glucopyranosyl-6-cyclopentylamino -9-β-D-ribofuranosylpurine (78).

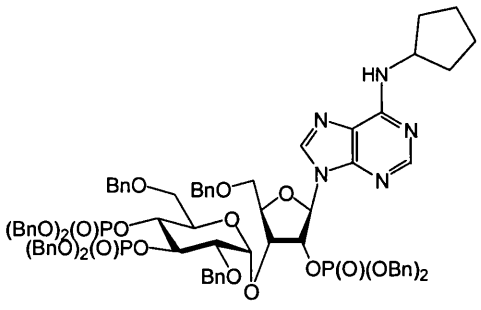


To a stirred solution of **77** (140 mg, 0.19 mmol) in ethanol (10 mL), cyclopentylamine (0.04 mL, 0.38 mmol) and triethylamine (0.03 mL, 0.23 mmol) were added. The mixture was heated at 80 °C for 3 h, after which time it was cooled and concentrated under reduced pressure. Water (20 mL) was added to the residue and was extracted with ethyl acetate (3 × 25 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave

an oil, which was purified by flash chromatography on silica using ethyl acetate-hexane (8:2) ethyl acetate-methanol (10:1) to give the title compound (80 mg, 54%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.44 (s, 1 H, H-8), 8.03 (s, 1 H, H-2), 7.37–7.21 (m, 15 H, ArC*H*), 6.28 (d, 1 H, $J_{1',2'}$ 5.9 Hz, H-1'), 6.18 (broad s, 1 H, N*H*), 5.92 (broad s, 1 H, O*H*), 4.77 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.66 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.58–4.42 (m, 7 H, 2.5 × OCH_2Ar, H-2', H-3'), 4.19–4.06 (m, 2 H, H-4', H-3''), 3.99–3.96 (m, 1 H, H-5''), 3.78–3.63 (m, 3 H, H-4'', H-6''a, H-6''b), 3.61–3.50 (m, 2 H, H-5'a, H-5'b), 3.40 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 9.8 Hz, H-2''), 2.3 (broad s, 2 H, O*H*), 2.17–2.09 (m, 2 H, cyclopentyl ring), 1.79–1.63 (m, 4 H, cyclopentyl ring) and 1.59–1.25 (m, 3 H, cyclopentyl ring); ^{13}C NMR (100 MHz; $CDCl_3$) 154.46 (C-2), 152.61 (C-4), 137.82, 137.72, 137.33 (3 × C-1 Bn ether ring), 136.60 (C-8), 128.67,128.54,128.40, 128.32, 128.19, 127.78, 127.55, 127.52 (Ar*C*H), 119.70 (C-5), 99.65 (C-1''), 87.63 (C-1'), 83.03, 79.23, 76.23 (3 × CH), 73.89, 73.61, 73.61 (3 × O*C*H_2Ar), 73.58, 72.75 (2 × CH), 72.17 (C-5' or C-6''), 69.96 (C-5' or C-6''), 69.60 (CH), 60.37 (N*C*H, cyclopentyl ring) 33.47 (2 × NCH*C*H_2 of cyclopentyl ring) and 23.81 (2 × NCHCH$_2$*C*H$_2$ of cyclopentyl ring); MS: (FAB) *m/z* 768.2 [(M+H)$^+$, 88%], *m/z* calcd for $C_{42}H_{49}N_5O_9$ [M+H]$^+$, 768.3608 found *m/z* 768.3612:

2'',5',6''-Tri-*O*-benzyl-2',3'',4''-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 6- cyclopentylamino -9-β-*D*- ribofuranosylpurine (79).

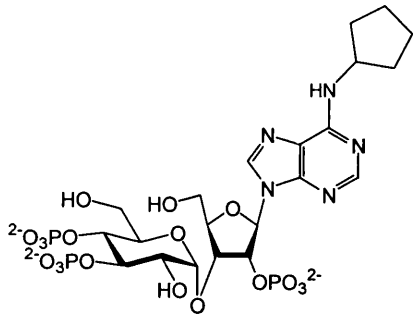


Bis(benzyloxy)(diisopropylamino)phosphine (220 mg, 0.6 mmol) and 1*H*-tetrazole (65 mg, 0.9 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **78** (80 mg, 0.1mmol). After a further 20 min, TLC (ethyl acetate:methanol, 95:5) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (180 mg, 0.6 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and ethyl acetate (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat.aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced

pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate-hexane (1:1 then 4:1 then 1:0) to give the title compound (40 mg, 25%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.48 (s, 1 H, H-8), 8.00 (s, 1 H, H-2), 7.63 (d, 1 H, *J* 8.9 Hz, N*H*), 7.38–6.95 (m, 45 H, ArC*H*), 6.29(d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 5.57–5.52 (m, 1 H, H-2') 5.30 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.00–4.85 (m, 8 H, H-3'', 3.5 × OCH_2Ar), 4.76–4.26 (m, 14 H, H-3', H-4', H-4'', 5.5 × OCH_2Ar) 3.82–3.80 (m, 1 H, H-5''), 3.68–3.48 (m, 5 H, H-5'a, H-5'b, H-2'', H-6''a, H-6''b) and 2.19–1.20 (m, 9 H, cyclopentyl ring); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 0.15, –0.66, –0.79; MS: (FAB) *m/z* 1564.8 [$(M+H)^+$, 44%], *m/z* calcd for $C_{84}H_{89}N_5O_{18}P_3$ $[M+H]^+$ 1564.5364, found *m/z* 1564.5345:

3'-O-*α*-*D*-Glucopyranosyl-6-cyclopentylamino-9-*β*-*D*-ribofuranosylpurine 2',3'',4''-trisphosphate (80).

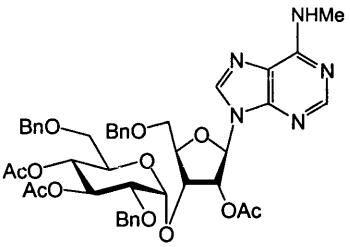


A mixture of **79** (40 mg, 0.025 mmol) and $Pd(OH)_2$ on carbon (10%, 110 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 65 °C for 3 h. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure to give the title trisphosphate (0.012 mmol, 46%) as its sodium salt.

^{1}H NMR (400 MHz; D_2O) δ_H 8.02 (s, 1 H, H-8), 8.00 (s, 1 H, H-2), 6.04 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.15 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1''), 5.08–5.03 (m, 1 H, H-2'), 4.55–4.41 (m, 1 H, H-3'), 4.32–4.21 (m, 3 H, H-4', H-4'', N*H*), 3.83–3.44 (m, 7 H, H-5'a, H-5'b, H-2'', H-3'', H-5'', H-6''a, H-6''b) and 1.99–1.42 (m, 9 H, cyclopentyl ring); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.28, 2.56 and 1.59; MS: (FAB) *m/z* 736.2 $[(M+H)^+$, 44%], *m/z* calcd for $C_{21}H_{34}N_5O_{18}P_3$ $[M–H]^-$ 736.1033, found *m/z* 736.1019:

7.5.5 Synthesis of 6-methylamino adenophostin

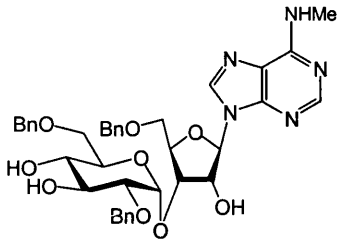
2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-methylamino-9-β-*D*-ribofuranosylpurine (81).



Methylamine hydrochloride (218 mg, 3.2 mmol) was added to a solution of **73** (390 mg, 0.46 mmol), triethylamine (0.9 mL, 6.4 mmol) in dichloromethane (5 mL) and ethanol (1 mL). The mixture was heated at 60 °C overnight, after which it was cooled and concentrated under reduced pressure. It was extracted with ethyl acetate (30 mL) and washed with water (20 mL). The extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using dichloromethane–acetone (40:1) as eluent to give the title compound (260 mg, 67%) as a colourless oil.

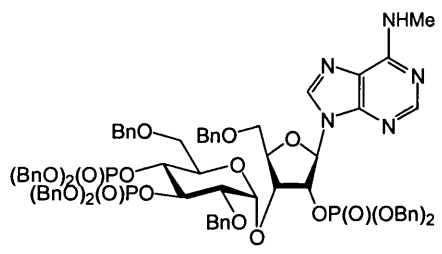
$[\alpha]^{20}_D$ +40.0 (*c* 0.60, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.41 (s, 1 H, H-8), 8.04 (s, 1 H, H-2), 7.40–7.23 (m, 15 H, ArC*H*), 6.30 (d, 1 H, $J_{1',2'}$ 5.1 Hz, H-1'), 5.87 (broad s, 1 H, N*H*), 5.71 (t, 1 H, $J_{2',1'}=J_{2',3'}$ 5.1 Hz, H-2'), 5.42 (t , 1 H, $J_{3",2"}=J_{3",4"}$ 9.8 Hz, H-3"), 5.03 (t, 1 H, $J_{4",3"}=J_{4",5"}$ 9.8 Hz, H-4"), 4.97 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.75 (t, 1 H, $J_{3',2'}=J_{3',4'}$ 5.1 Hz, H-3'), 4.61 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.56–4.44 (m, 5 H, H-4', 2 × OCH_2Ar), 4.41 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 3.99–3.95 (m, 1 H, H-5"), 3.74 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5'a), 3.64 (dd, 1 H, $J_{5'b,4'}$ 3.1 Hz, $J_{5'b,5'a}$ 10.9 Hz, H-5'b), 3.54 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.8 Hz, H-2"), 3.39–3.33 (m, 2 H, H-6"a, H-6"b), 3.19 (broad s, 3 H, NCH_3), 1.97 (s, 3 H, CH_3CO), 1.93 (s, 3 H, CH_3CO) and 1.88 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz; $CDCl_3$) 170.05, 169.95, 169.51 (3 × O*C*OCH$_3$), 155.24 (C-2), 153.21 (C-4), 153.20 (C-6), 138.00 (C-8), 137.43, 137.26, 137.20 (3 × C-1 Bn ether ring), 128.41, 138.32, 127.82, 127.79, 127.64, 127.60, 127.56 (Ar*C*H), 97.90 (C-1"), 86.07 (C-1'), 82.38, 76.69, 76.59, 76.33 (4 × CH), 74.25, 73.58, 73.47 (3 × O*C*H$_2$Ar), 73.24 (CH), 71.78 (2 × CH), 69.15, 68.94 (C-5', C-6") 67.75 (CH), 29.34 (N*C*H$_3$) and 20.98, 20.80, 20.53 (3 × CH$_3$*C*O); MS: (FAB) *m/z* 840.3 [(M+H)$^+$, 42%], *m/z* calcd for $C_{44}H_{49}N_5O_{12}$ [M+H] 840.3455 found *m/z* 840.3469:

2",5',6"-Tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-methylamino-9-β-*D*-ribofuranosylpurine (82).



NaOMe (5 mg, cat) was added to a solution of **81** (100 mg, 0.12 mmol) and the mixture was stirred for 30 min. It was then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil (80 mg, 94%) which was crystallised from methanol. m.p.174–175 °C; $[\alpha]^{20}_D$ 0.00 ± 1 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.31 (s, 1 H, H-8), 8.02 (s, 1 H, H-2), 7.49–7.22 (m, 15 H, ArC*H*), 6.25 (d, 1 H, $J_{1',2'}$ 5.9 Hz, H-1'), 6.09 (broad s, 1 H, N*H*), 5.67 (broad s, 1 H, O*H*), 4.80 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.70 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.68–4.38 (m, 7 H, H-2', H-3', 2.5 × OCH_2Ar), 4.19–4.18 (m, 1 H, H-4'), 4.11 (t , 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.7 Hz, H-3"), 3.96–3.90 (m, 1 H, H-5"), 3.78–3.60 (m, 3 H, H-4", H-6"a, H-6"b), 3.58–3.47 (m, 2 H, H-5'a, H-5'b), 3.42 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.7 Hz, H-2") and 3.18 (broad s, 3 H, NCH_3); ^{13}C NMR (100 MHz; $CDCl_3$) 155.32 (C-6, C-2), 152.64 (C-4), 137.88, 137.76, 137.30 (3 × C-1 Bn ether ring), 136.64 (C-8), 128.63, 128.55, 128.40, 128.33, 128.22, 127.78, 127.55 (Ar*C*H), 119.87 (C-5), 99.50 (C-1"), 87.82 (C-1'), 86.63, 83.03, 79.12, 76.02 (4 × CH), 73.90, 73.62 (3 × O*C*H_2Ar), 73.43, 72.81 (2 × CH), 71.98 (C-5' or C-6"), 69.93 (CH), 69.56 (C-5' or C-6"), and 48.05 (N*C*H_3); MS: (FAB) *m/z* calcd for $C_{38}H_{43}N_5O_9$ $[M+H]^+$ 714.3139 found *m/z* 714.3139. Anal. Calcd for $C_{38}H_{43}N_5O_9$ C, 63.94; H, 6.07; N 9.81%. Found: C, 63.60; H, 6.03; N 9.59%:

2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 6- methylamino -9-β-*D*- ribofuranosylpurine (83).

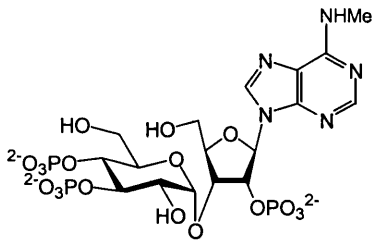


Bis(benzyloxy)(diisopropylamino)phosphine (232 mg, 0.67 mmol) and 1*H*-tetrazole (71 mg, 1.01 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **82** (80 mg, 0.1 mmol). After a further 20 min, TLC (ethyl acetate:methanol, 95:5) indicated conversion of starting material to a

single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (192 mg, 0.67 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and ethyl acetate (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (1:1 then 4:1) to give the title compound (60 mg, 36%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.31 (s, 1 H, H-8), 7.83 (s, 1 H, H-2), 7.52–6.95 (m, 45 H, ArC*H*), 6.32 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H-1'), 5.73 (br s, 1 H, NH), 5.65–5.60 (m, 1 H, H-2'), 5.32 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.05–4.86 (m, 8 H, H-3'', 3.5 × OCH_2Ar), 4.76–4.36 (m, 13 H, H-3', H-4', H-4'', 5 × OCH_2Ar), 4.30 (AB, 1 H, 0.5 × OCH_2Ar), 3.84–3.82 (m, 1 H, H-5''), 3.66–3.53 (m, 5 H, H-5'a, H-5'b, H-2'', H-6''a, H-6''b) and 3.16 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P –0.24, –0.91, –1.05; MS: (FAB) *m/z* 1494.8 [$(M+H)^+$, 84%], *m/z* calcd for $C_{80}H_{82}N_5O_{18}P_3$ $[M+H]^+$ 1494.4946 found *m/z* 1494.4942:

3'-O-*α*-*D*-Glucopyranosyl-6-methylamino-9-*β*-*D*-ribofuranosylpurine 2',3'',4''-trisphosphate (84).



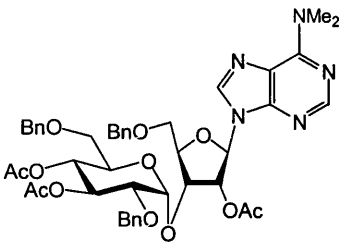
A mixture of **83** (28 mg, 0.018 mmol) and $Pd(OH)_2$ on carbon (10%, 45 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 65 °C for 3 h. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure to give the title trisphosphate (0.016 mmol, 80%) as its sodium salt.

^{1}H NMR (400 MHz; D_2O) δ_H 8.09 (s, 2 H, H-2, H-8), 6.12 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.20 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1''), 5.17–5.11 (m, 1 H, H-2'), 4.49–4.48 (m, 1 H, H-3'), 4.38–4.30 (m, 3 H, H-4', H-3'', N*H*), 3.87 (m, 1 H, H-4''), 3.78–3.59 (m, 6 H, H-5'a, H-5'b, H-2'', H-5'', H-6''a, H-6''b) and 2.92 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; D_2O; ^{1}H

decoupled) δ_P 2.45, 2.10 and 0.86; MS: (FAB) *m/z* 682.1 [$(M-H)^-$, 84%], *m/z* calcd for $C_{17}H_{28}N_5O_{18}P_3$ $[M-H]^-$ 682.0564 found *m/z* 682.0567:

7.5.6 Synthesis of 6-dimethylamino adenophostin

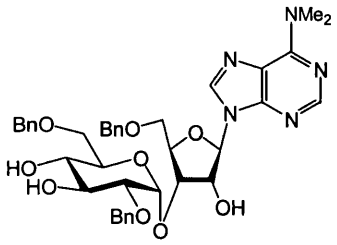
2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-dimethylamino-9-β-*D*-ribofuranosylpurine (85).



Dimethylamine hydrochloride (180 mg, 2.20 mmol) was added to a solution of **73** (300 mg, 0.35 mmol), triethylamine (0.62 mL, 4.4 mmol) in dichloromethane (5 mL) and ethanol (1 mL). The mixture was heated at 60 °C 2 h, after which it was cooled and concentrated under reduced pressure. It was extracted with ethyl acetate (30 mL) and washed with water (20 mL). The extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using dichloromethane-acetone (50:1 then 9:1) as eluent to give the title compound (290 mg, 96%) as a colourless oil.

$[\alpha]^{20}_D$ +52.9 (*c* 0.55,$CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.46 (s, 1 H, H-8), 8.17 (s, 1 H, H-2), 7.47–7.17 (m, 15 H, ArC*H*), 6.44 (d, 1 H, $J_{1',2'}$ 5.1 Hz, H-1'), 5.82 (t, 1 H, $J_{2',1'}$ =$J_{2',3'}$ 5.1 Hz, H-2'), 5.53 (t , 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.8 Hz, H-3"), 5.16 (t, 1 H, $J_{4",3"}$= $J_{4",5"}$ 9.8 Hz, H-4"), 5.08 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.85 (t, 1 H, $J_{3',2'}$ =$J_{3',4'}$ 5.1 Hz, H-3'), 4.74 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.70–4.57 (m, 5 H, H-4', 2 × OCH_2Ar), 4.43 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.11–4.06 (m, 1 H, H-5"), 3.85 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5'a), 3.75 (dd, 1 H, $J_{5'b,4'}$ 3.1 Hz, $J_{5'b,5'a}$ 10.9 Hz, H-5'b), 3.67–3.64 (m, 7 H, H-2", N(CH_3)$_2$), 3.52–3.44 (m, 2 H, H-6"a, H-6"b), 2.08 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO) and 1.99 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz; $CDCl_3$) 170.05, 169.92, 169.49 (3 × O*C*OCH$_3$), 154.68 (C-2), 152.39, 150.18 (C-4, C-6), 137.43 (C-8), 137.25, 137.24, 136.38 (3 × C-1 Bn ether ring), 128.36, 128.28, 12.8.18, 127.79, 127.71, 127.67, 127.61, 127.52 (Ar*C*H), 97.80 (C-1"), 85.93 (C-1'), 82.23, 76.57, 76.16, 76.33 (4 × CH), 74.13, 73.52, 73.43 (3 ×O*C*H$_2$Ar), 73.21, 71.77 (2 × CH), 69.09, 69.05 (C-5', C-6"), 68.93 (CH), 38.62, 31.01 (N*C*H$_3$) and 20.95, 20.77, 20.52 (3 × CH$_3$*C*O); MS: (FAB) *m/z* 854.2 [(M+H)$^+$, 48%], *m/z* calcd for $C_{45}H_{50}N_5O_{12}$ [M+H] 854.3567, found *m/z* 854.3619; Anal. Calcd for $C_{45}H_{50}N_5O_{12}$ C, 63.30; H, 5.90; N 8.27%. Found: C, 62.80; H, 5.30; N 7.97%:

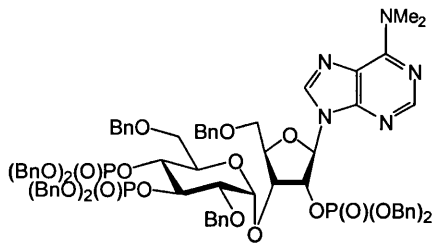
2",5',6"-Tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-dimethylamino-9-β-*D*-ribofuranosylpurine (86).



NaOMe (30 mg, cat) was added to a solution of **85** (290 mg, 0.34 mmol) in methanol and the mixture was stirred for 30 min. It was then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil which was crystallised from methanol (140 mg, 57%).

m.p.153–155 °C; $[\alpha]^{20}_D$ +2.2 ± 1 (*c* 0.45,$CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.26 (s, 1 H, H-8), 8.02 (s, 1 H, H-2), 7.35–7.24 (m, 15 H, ArC*H*), 6.20 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 4.85 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.76–4.38 (m, 8 H, H-2', H-3', 3 × OCH_2Ar), 4.25–4.23 (m, 1 H, H-4'), 4.08 (t , 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.8 Hz, H-3"), 3.91–3.88 (m, 1 H, H-5"), 3.69–3.49 (m, 11 H, H-5'a, H-5'b, H-4", H-6"a, H-6"b, N(CH_3)$_2$) and 3.41 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.8 Hz, H-2"); ^{13}C NMR (100 MHz; $CDCl_3$) 154.77 (C-6), 151.72 (C-2), 150.00 (C-4), 137.76, 137.34, 136.55 (3 × C-1 Bn ether ring), 136.26 (C-8), 128.74, 128.59, 128.41, 128.23, 127.75, 127.58, 127.55, 127.52 (Ar*C*H), 120.19 (C-5), 99.55 (C-1"), 87.48 (C-1'), 83.07, 80.47, 79.09, 76.25 (4 × CH), 73.84, 73.59, 73.56 (3 ×OCH_2Ar), 72.72 (CH), 71.99 (CH), 69.91 (C-5' or C-6"), 69.64 (CH), 69.54 (C-5' or C-6"), 38.86, (NCH_3); MS: (FAB) *m/z* calcd for $C_{39}H_{45}N_5O_9$ $[M+H]^+$ 728.3250 found *m/z* 728.3280:

2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 6-dimethylamino -9-β-*D*- ribofuranosylpurine (87).

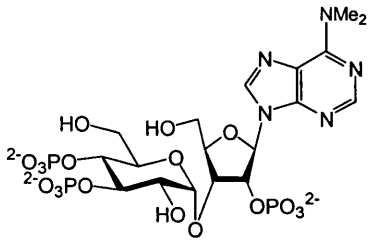


Bis(benzyloxy)(diisopropylamino)phosphine (110 mg, 0.3 mmol) and 1*H*-tetrazole (35 mg, 0.5 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **86** (40 mg, 0.05 mmol). After a further 20 min, TLC (ethyl acetate:methanol, 95:5) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (206 mg, 0.72 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and ethyl acetate (20 mL) were added and the mixture was allowed to heat up to room temperature.

The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (1:1 then 4:1) to give the title compound (45 mg, 54%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.26 (s, 1 H, H-8), 7.89 (s, 1 H, H-2), 7.39–6.95 (m, 45 H, ArC*H*), 6.37 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 5.69–5.64 (m, 1 H, H-2'), 5.32 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.09 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OC*H*$_2$Ar), 5.06–4.85 (m, 10 H, H-3'', 4.5 × OC*H*$_2$Ar), 4.47–4.36 (m, 10 H, H-3', H-4', H-4'', 3.5 × OC*H*$_2$Ar), 4.29 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OC*H*$_2$Ar), 3.84–3.82 (m, 1 H, H-5'') and 3.65–3.43 (m, 11 H, H-5'a, H-5'b, H-2'', H-6''a, H-6''b, N(C*H*$_3$)$_2$); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P –0.05, –0.66, –0.82; MS: (FAB) *m/z* 1508.4 [(M+H)$^+$, 79%], *m/z* calcd for $C_{81}H_{84}N_5O_{18}P_3$ [M+H]$^+$ 1508.5102 found *m/z* 1508.5068:

3'-O-α-D-Glucopyranosyl-6-methylamino-9-β-D-ribofuranosylpurine 2',3'',4''-trisphosphate (88).

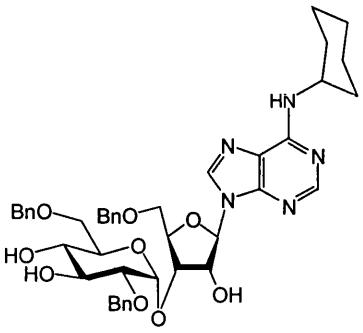


A mixture of **87** (20 mg, 0.013 mmol) and $Pd(OH)_2$ on carbon (10%, 50 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 70 °C for 2 hours. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure and purified by HPLC to give the title trisphosphate (4 μmol, 31%) as the free acid.

^{1}H NMR (400 MHz; D_2O) δ_H 8.29 (s, 1 H, H-8), 8.17 (s, 1 H, H-2), 6.19 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.12 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1''), 5.10–5.05 (m, 1 H, H-2'), 4.46–4.44 (m, 1 H, H-3'), 4.34–4.27 (m, 2 H, H-4', H-3''), 3.93–3.86 (m, 1 H, H-4'') and 3.71–3.56 (m, 12 H, H-5'a, H-5'b, H-2'', H-5'', H-6''a, H-6''b, N(C*H*$_3$)$_2$); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 4.61 2 × P and 4.52; MS: (FAB) *m/z* 698.0 [(M+H)$^+$, 40%], *m/z* calcd for $C_{18}H_{30}N_5O_{18}P_3$ [M+H]$^+$ 698.0877 found *m/z* 698.0894:

7.5.7 Synthesis of 6-cyclohexylamino adenophostin

2',5',6"-Tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-6-cyclohexylamino-9-β-*D*-ribofuranosylpurine (90).

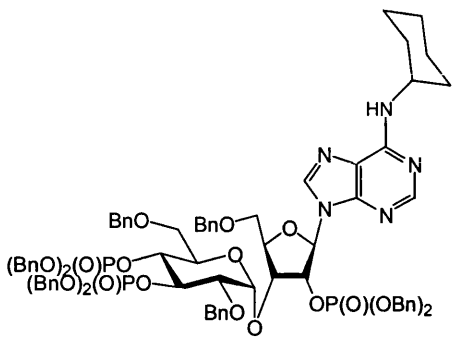


Cyclohexylamine (0.08 mL, 1.06 mmol) was added to a solution of **73** (150 mg, 0.17 mmol), triethylamine (0.15 mL, 1.06 mmol) in dichloromethane (5 mL) and ethanol (1 mL). The mixture was heated at 60 °C overnight, after which it was cooled and concentrated under reduced pressure. It was extracted with ethyl acetate (30 mL) and washed with water (20 mL). The extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil. The oil was dissolved in methanol and NaOMe (30 mg, cat) was added and the mixture was stirred for 30 min. It was the concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil which was crystallised from methanol (70 mg, 50%).

m.p.103–106 °C; $[\alpha]^{20}_D$ +0.01 ± 1 (*c* 0.38,$CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.26 (s, 1 H, H-8), 8.01 (s, 1 H, H-2), 7.37–7.22 (m, 15 H, ArC*H*), 6.25 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 5.85 (broad s, 2 H, N*H*, O*H*), 4.78 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.65 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.59–4.38 (m, 7 H, H-2', H-3', 2.5 × OCH_2Ar), 4.17–4.09 (m, 3 H, H-4', H-3", O*H*), 3.97–3.94 (m, 1 H, H-5"), 3.76–3.64 (m, 3 H, H-4", H-6"a, H-6"b), 3.58–3.51 (m, 2 H, H-5'a, H-5'b), 3.47 (d, 1 H, *J* 5.1 Hz, cyclohexyl), 3.41 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.9 Hz, H-2"), 2.11–1.91 (m, 3 H, cyclohexyl), 1.91–1.77 (m, 2 H, cyclohexyl), 1.68–1.66 (m, 1 H, cyclohexyl), 1.52–1.42 (m, 2 H, cyclohexyl) and 1.34–1.22 (m, 2 H, cyclohexyl); ^{13}C NMR (100 MHz; $CDCl_3$) 154.33 (C-2), 152.94 (C-4, C-6), 138.09 (C-8), 137.95, 137.59, 136.84 (3 × C-1 Bn ether ring), 129.06, 128.90, 128.77, 128.72, 128.54, 128.16, 127.94, 127.89 (Ar*C*H), 119.87 (C-5), 99.97 (C-1"), 87.82 (C-1'), 83.43, 82.06, 81.05, 79.53, 76.74 (5 × CH), 74.22, 73.97, 73.92 (3 ×OCH_2Ar), 73.01, 72.46 (2 × CH), 70.31 (*C*H_2), 69.91 (*C*H_2 and *C*H), 33.68, 26.04 and 25.33 (3 × *C*H_2); MS: (FAB) *m/z* 782.3 [$(M+H)^+$, 60%], *m/z* calcd for $C_{43}H_{51}N_5O_9$ $[M+H]^+$, 782.3765

found *m/z* 782.3768. Anal. Calcd for $C_{43}H_{51}N_5O_9$ C, 66.05; H, 6.57; N 8.96%. Found: C, 65.7; H, 6.52; N 8.83%:

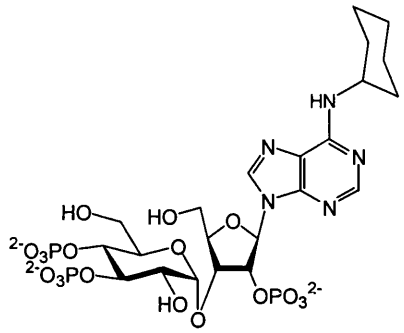
2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 6-cyclohexylamino -9-β-*D*- ribofuranosylpurine (91).



Bis(benzyloxy)(diisopropylamino)phosphine (110 mg, 0.3 mmol) and 1*H*-tetrazole (32 mg, 0.46 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **90** (40 mg, 0.05 mmol). After a further 20 min, TLC (ethyl acetate) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (103 mg, 0.35 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and dichloromethane (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (2:3 then 1:1 then 4:1) to give the title compound (55 mg, 68%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.28 (s, 1 H, H-8), 7.82 (s, 1 H, H-2), 7.39–6.94 (m, 45 H, ArC*H*), 6.32 (d, 1 H, $J_{1',2'}$ 6.6 Hz, H-1'), 5.69 (br s, 1 H, NH), 5.64–5.59 (m, 1 H, H-2'), 5.31 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 5.08–4.30 (m, 21 H, H-3', H-4', H-3", H-4", 8.5 × OCH_2Ar,), 4.30 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 3.89–3.81 (m, 1 H, H-5"), 3.66–3.23 (m, 5 H, H-5'a, H-5'b, H-2", H-6"a, H-6"b) and 2.18–1.18 (m, 11 H, cyclohexyl); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P –0.23, –0.89, –1.05; MS: (FAB) *m/z* 1563.6 [$(M+H)^+$, 64%], *m/z* calcd for $C_{85}H_{90}N_5O_{18}P_3$ $[M+H]^+$ 1563.5605 found *m/z* 1563.5602:

3'-O-α-D-Glucopyranosyl-6-cyclohexylamino-9-β-D-ribofuranosylpurine 2',3",4"-trisphosphate (92).

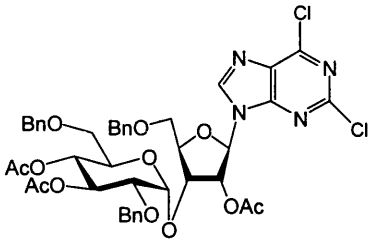


A mixture of **91** (25 mg, 0.016 mmol) and $Pd(OH)_2$ on carbon (10%, 45 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 70 °C for 2 hours. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated under reduced pressure to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure to give the title trisphosphate (15.27 μmol, 95%) as the sodium salt.

^{1}H NMR (400 MHz; D_2O) δ_H 8.07 (s, 1 H, H-8), 8.03 (s, 1 H, H-2), 6.09 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 5.19 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 5.14–5.08 (m, 1 H, H-2'), 4.59–4.49 (m, 1 H, H-3'), 4.35–4.26 (m, 3 H, H-4', H-3", H-4") and 3.89–3.58 (m, 6 H, H-5'a, H-5'b, H-2", H-5", H-6"a, H-6"b), 1.89–1.82 (m, 2 H, cyclohexyl), 1.73–1.59 (m, 2 H, cyclohexyl), 1.49–1.45 (m, 1 H, cyclohexyl) and 1.35–1.08 (m, 6 H, cyclohexyl); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.31, 2.66 and 1.49; MS: (FAB) *m/z* 752.1 $[(M+H)^+$, 60%], *m/z* calcd for $C_{22}H_{36}N_5O_{18}P_3$ $[M+H]^+$ 752.1346 found *m/z* 752.1361:

7.5.8 Synthesis of 2,6 disubstituted compounds

2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-2,6-dichloro-9-β-*D*-ribofuranosylpurine (93).

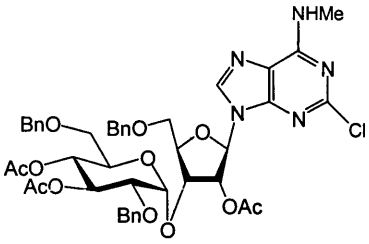


TMSOTf (0.6 mL, 3.3 mmol) was added cautiously dropwise to a stirred solution of **56** (500 mg, 0.66 mmol), 2,6-dichloropurine (138 mg, 0.73 mmol) and DBU (0.4 mL, 2.67 mmol) in acetonitrile (5 mL) at 0 °C. The mixture was heated at 60 °C for 1 h, after which it was cooled and quenched by careful addition of sat. aq. $NaHCO_3$ solution (25 mL). The mixture was extracted with dichloromethane (3 × 30 mL) and the combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave a yellow oil. Purification by flash chromatography on silica using ethyl acetate–hexane (3:7) as eluent gave the title compound (390 mg, 67%) as a colourless oil.

$[\alpha]^{20}_D$ +63.3 (*c* 0.44, $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$) δ_H 8.45 (s, 1 H, H-8), 7.37–7.16 (m, 15 H, ArC*H*), 6.37 (d, 1 H, $J_{1',2'}$ 5.6 Hz, H-1'), 5.61 (t, 1 H, $J_{2',1'}=J_{2',3'}$ 5.6 Hz, H-2'), 5.45 (t, 1 H, $J_{3",4"}=J_{3",2"}$ 9.8 Hz, H-3"), 5.02 (t, 1 H, $J_{4",3"}=J_{4",5"}$ 9.8 Hz, H-4"), 4.97 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.69 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, $J_{3',2'}$ 5.6 Hz, H-3'), 4.63 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × O*CH*$_2$Ar), 4.56–4.42 (m, 5 H, 2 × O*CH*$_2$Ar, H-4'), 4.36 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × O*CH*$_2$Ar), 4.02–3.98 (m, 1 H, H-5"), 3.70 (dd, 1 H, $J_{5'a,4'}$ 2.4 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5'a), 3.60–3.55 (m, 2 H, H-2", H-5'b), 3.45–3.38 (m, 2 H, H-6"a, H-6 " b), 1.99 (s, 3 H, C*H*$_3$CO), 1.96 (s, 3 H, C*H*$_3$CO) and 1.89 (s, 3 H, C*H*$_3$CO); ^{13}C NMR (100 MHz; $CDCl_3$) 169.88, 169.88, 169.43 (3 × O*C*OCH$_3$), 152.86 (C-4 or C-6), 152.51 (C-2), 151.48 (C-4 or C-6), 144.02 (C-8), 137.23, 137.13, 136.59 (3 × C-1 Bn ether ring), 130.81 (C-5), 128.45, 128.27, 128.14, 127.97, 127.82, 127.71, 127.60 (Ar*C*H), 98.12 (C-1"), 86.43 (C-1'), 83.46, 76.68, 76.49, 74.96, 74.59 (5 × CH), 73.64, 73.43, 73.15 (3 × O*C*H$_2$Ar), 71.61, 69.25 (2 × CH), 67.11 (C-5' or C-6"), 68.91 (CH), 67.96 (C-5' or C-6"), 20.89, 20.70, 20.33 (3 × CH$_3$*C*O); MS: (FAB) *m/z* 879.1 [(M+H)$^+$, 83%], *m/z* calcd for $C_{43}H_{43}N_4O_{12}Cl_2$ [M+H]$^+$ $^{37}Cl_2$ 883.2352 found *m/z* 883.2394, [M+H]$^+$ ^{35}Cl,^{37}Cl 881.2381 found *m/z* 881.2391, [M+H]$^+$ $^{35}Cl_2$ 879.2411 found *m/z* 879.2400; Anal. Calcd for $C_{43}H_{43}N_4O_{12}Cl_2$ C, 58.64; H, 5.15; N 6.36%. Found: C, 58.80; H, 5.09; N 5.94%:

7.5.8.1 Synthesis of 2-methoxy-6-methylamino Adenophostin

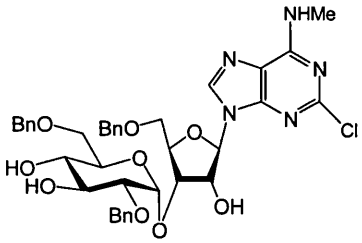
2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-2-chloro-6-methylamino-9-β-*D*-ribofuranosylpurine (94).



Methylamine hydrochloride (179 mg, 2.6 mmol) was added to a solution of **93** (390 mg, 0.44 mmol), triethylamine (0.74 mL, 5.3 mmol) in dichloromethane (5 mL) and ethanol (1 mL). The mixture was heated at 60 °C for 4 h, after which it was cooled and concentrated under reduced pressure. It was extracted with ethyl acetate (30 mL) and washed with water (20 mL). The extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using dichloromethane–acetone (40:1) as eluent to give the title compound (260 mg, 67%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.99 (s, 1 H, H-8), 7.36–7.24 (m, 15 H, ArC*H*), 6.27 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1'), 6.16 (broad s, 1 H, N*H*), 5.61 (t, 1 H, $J_{2',1'}$ =$J_{2',3'}$ 5.5 Hz, H-2'), 5.43 (dd , 1 H, $J_{3",2"}$ 10.1, $J_{3",4"}$ 9.4 Hz, H-3"), 5.05 (t, 1 H, $J_{4",3"}$= $J_{4",5"}$ 9.4 Hz, H-4"), 4.97 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.71 (t, 1 H, $J_{3',2'}$ =$J_{3',4'}$ 5.5 Hz, H-3'), 4.62 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.55–4.47 (m, 5 H, H-4', 2 × OCH_2Ar), 4.34 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.00–3.96 (m, 1 H, H-5"), 3.63 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5'a), 3.55 (dd, 1 H, $J_{5'b,4'}$ 3.1 Hz, $J_{5'b,5'a}$ 10.5 Hz, H-5'b), 3.40 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 10.1 Hz, H-2"), 3.36–3.26 (m, 2 H, H-6"a, H-6"b), 3.16 (broad s, 3 H, NCH_3), 1.98 (s, 3 H, CH_3CO), 1.94 (s, 3 H, CH_3CO) and 1.89 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz; $CDCl_3$) 170.1, 169.9, 169.5 (3 × O*C*OCH$_3$), 155.7, 149.4 (C-6, C-4), 138.3 (C-8), 137.4, 137.3, 137.2 (3 × C-1 Bn ether ring), 132.8 (C-2), 128.4, 128.2, 127.8, 127.7, 127.6, 127.5, (Ar*C*H), 118.9 (C-5), 98.0 (C-1"), 85.9 (C-1'), 82.7, 76.5, 74.5 (3 × CH), 73.6, 73.4, 73.1 (3 ×O*C*H$_2$Ar), 71.7 (CH), 69.3 (C-5' or C-6"), 69.9, 68.9 (2 × CH), 67.8 (C-5' or C-6"), 31.0 (N*C*H$_3$), 20.9, 20.8, 20.5 (3 × CH$_3$*C*O); MS: (FAB) *m/z* 874.1 [(M+H)$^+$, 90%], *m/z* calcd for $C_{44}H_{47}N_5O_{12}Cl$ [M+H]$^+$ ^{37}Cl 876.2992 found *m/z* 876.3042, [M+H]$^+$ ^{35}Cl 874.3021 found *m/z* 874.3043:

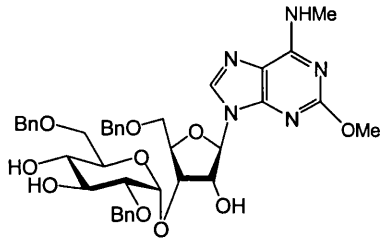
2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-2-chloro-6-methylamino-9-β-*D*-ribofuranosylpurine (95).



NaOMe (10 mg, 0.16 mmol) was added to a solution of **94** (70 mg, 0.08 mmol) in methanol and the mixture was stirred at 65 °C overnight. It was then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil (50 mg, 84%).

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.97 (s, 1 H, H-8), 7.37–7.21 (m, 15 H, ArC*H*), 6.28 (br s, 1 H, N*H*), 6.22 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 4.73 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.65 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar) 4.50–4.38 (m, 7 H, H-2', H-4', 2.5 × OCH_2Ar), 4.16–4.11 (m, 3 H, H-3', H-3", H-5"), 3.92–3.87 (m, 1 H, H-6"a), 3.74 (t, 1 H, $J_{3",2"}$ =$J_{3",4"}$ 10.0 Hz, H-4"), 3.66 (dd, 1 H, $J_{6"b,5"}$ 3.1 Hz, $J_{6"b,6"a}$ 10.5 Hz, H-6"b), 3.53–3.48 (m, 2 H, H-5'a, H-5'b), 3.41 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 10.0 Hz, H-2") and 3.14 (s, 3 H, NCH_3); MS: (FAB) *m/z* 748.2 [(M+H)$^+$, 36%], *m/z* calcd for $C_{38}H_{42}N_5O_9Cl$ [M+H]$^+$ ^{37}Cl 750.2719 found *m/z* 750.2754, [M+H]$^+$ ^{35}Cl 748.2749 found *m/z* 748.2759:

2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-2-methoxy-6-methylamino-9-β-*D*-ribofuranosylpurine (96).

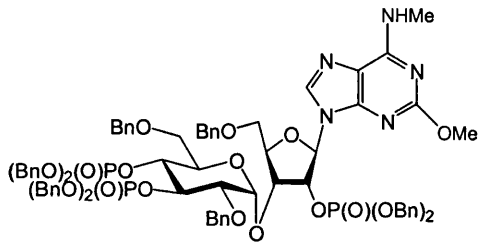


NaOMe (61 mg, 1.13 mmol) was added to a solution of **95** (100 mg, 0.11 mmol) in methanol (10 mL) and the mixture was heated at reflux overnight. It was the concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil which was purified by flash chromatography on silica using dichloromethane–acetone (40:1) as eluent to give the title compound which was crystallised from methanol (60 mg, 71%).

m.p.192–193 °C; $[\alpha]^{20}_D$ +0.00 ± 1 (*c* 0.40, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.79 (s, 1 H, H-8), 7.37–7.22 (m, 15 H, ArC*H*), 6.10 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 5.93 (br s, 1 H, N*H*), 5.06 (br s, 1 H, OH), 4.80 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.70 (AB, 1 H, J_{AB} 11.7

Hz, 0.5 × OCH_2Ar), 4.60–4.42 (m, 6 H, H-2', H-3', 2 × OCH_2Ar), 4.70 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.31–4.29 (m, 1 H, H-4'), 3.74 (t , 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.8 Hz, H-3"), 3.97–3.94 (m, 2 H, H-5", OH), 3.79 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'b,5'a}$ 10.5 Hz, H-5'a), 3.68–3.61 (m, 4 H, H-5'b, H-4", H-6"a, H-6"b), 3.86 (s, 3 H, OCH_3), 3.42 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.8 Hz, H-2") and 3.14 (s, 3 H, NCH_3); MS: (FAB) *m/z* 744.2 $[(M+H)^+$, 61%], *m/z* calcd for $C_{39}H_{45}N_5O_{18}$ $[M+H]^+$ ^{37}Cl 744.3244 found *m/z* 744.3246; Anal. Calcd for $C_4H_4N_5O_{12}$ C, 62.98; H, 6.10; N 9.42%. Found: C, 62.50; H, 6.10; N 9.25%:

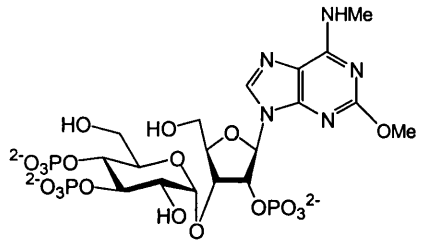
2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 2-methoxy-6-methylamino -9-β-*D*-ribofuranosylpurine (97).



Bis(benzyloxy)(diisopropylamino)phosphine (83 mg, 0.24 mmol) and 1*H*-tetrazole (25 mg, 0.4 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **96** (30 mg, 0.04 mmol). After a further 20 min, TLC (ethyl acetate) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (81 mg, 0.28 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and ethyl acetate (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (2:3 then 1:1 then 4:1) to give the title compound (35 mg, 57%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.63 (s, 1 H, H-8), 7.37–6.96 (m, 45 H, ArC*H*), 6.37 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 5.82 (broad s, 1 H, N*H*), 5.71–5.61 (m, 1 H, H-2'), 5.33 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 5.02–4.87 (m, 8 H, H-3", 3.5 × OCH_2Ar), 4.77–4.27 (m, 14 H, H-3', H-4', H-4", 5.5 × OCH_2Ar), 3.86–3.80 (m, 1 H, H-5"), 3.70 (s, 3 H, OCH_3), 3.69–3.53 (m, 5 H, H-5'a, H-5'b, H-2", H-6"a, H-6"b) and 3.11 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 0.04, –0.58, –0.79; MS: (FAB) *m/z* 1524.1 [(M), 89%], *m/z* calcd for $C_{81}H_{84}N_5O_{18}P_3$ $[M+H]^+$ 1525.5085 found *m/z* 1525.5159:

3'-*O*-α-*D*-Glucopyranosyl-2-methoxy-6-methylamino-9-β-*D*-ribofuranosylpurine 2',3",4"-trisphosphate (98).

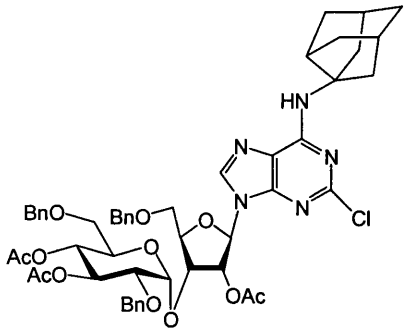


A mixture of **97** (20 mg, 0.013 mmol) and $Pd(OH)_2$ on carbon (10%, 50 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 70 °C for 2 h. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentratedunder reduced pressure to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure and purified by HPLC to give the title trisphosphate (6.06 μmol, 46%) as the free acid.

^{1}H NMR (400 MHz; D_2O) δ_H 7.82 (s, 1 H, H-8), 5.99 (d, 1 H, $J_{1',2'}$ 5.1 Hz, H-1'), 5.28–5.23 (m, 1 H, H-2'), 5.11 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.30–4.18 (m, 3 H, H-3', H-4', H-3"), 3.83–3.80 (m, 1 H, H-4"), 3.77 (s, 3 H, OCH_3), 3.74–3.31 (m, 6 H, H-5'a, H-5'b, H-2", H-5", H-6"a, H-6"b) and 3.12 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.15, 2.28 and 1.33; MS: (FAB) *m/z* 714.0 $[(M+H)^+$, 69%], *m/z* calcd for $C_{18}H_{30}N_5O_{18}P_3$ $[M+H]^+$ 714.0826 found *m/z* 714.0860:

7.5.8.2 Synthesis of 6-(3-noradamantylamino) adenophostin

2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-2-chloro-6-(-3-noradamantylamino)-9-β-*D*-ribofuranosylpurine (99).

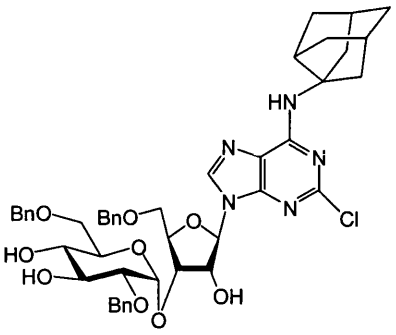


3-Noradamantanamine hydrochloride (212 mg, 1.2 mmol) was added to a solution of **93** (179 mg, 0.2 mmol) and triethylamine (0.34 mL, 2.4 mmol) in dichloromethane (5 mL) and ethanol (1 mL). The mixture was heated at 60 °C overnight, after which it was cooled and concentrated under reduced pressure. It was extracted with ethyl acetate (30 mL) and washed with water (20 mL). The extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography using ethyl acetate–hexane (4:1) as eluent to give the title compound (160 mg, 80%) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.97 (s, 1 H, H-8), 7.36–7.24 (m, 15 H, ArC*H*), 6.26 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1'), 6.16 (br s, 1 H, N*H*), 5.61 (dd, 1 H, $J_{2',1'}$ 5.5 Hz, $J_{2',3'}$ 5.0 Hz, H-2'), 5.43 (t, 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.9 Hz, H-3"), 5.04 (t, 1 H, $J_{4",3"}$=$J_{4",5"}$ 9.9 Hz, H-4"), 4.97 (d, 1 H, $J_{1",2"}$ 3.3 Hz, H-1"), 4.72 (t, 1 H, $J_{3',2'}$ =$J_{3',4'}$ 5.0 Hz, H-3'), 4.62 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.55–4.46 (m, 5 H, H-4', 2 × OCH_2Ar), 4.36 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.10–3.97 (m, 1 H, H-5"), 3.71 (dd, 1 H, $J_{5'a,4'}$ 2.9 Hz, $J_{5'a,5'b}$ 10.6 Hz, H-5'a), 3.63 (dd, 1 H, $J_{5'b,4'}$ 3.3 Hz, $J_{5'b,5'a}$ 10.6 Hz, H-5'b), 3.54 (dd, 1 H, $J_{2",1"}$ 3.3 Hz, $J_{2",3"}$ 9.9 Hz, H-2"), 3.43–3.35 (m, 2 H, H-6"a, H-6"b), 2.65–2.61 (m, 1 H, noradamantane), 2.35–2.19 (m, 7 H, noradamantane), 2.05–1.94 (m, 1 H, noradamantane), 1.98 (s, 3 H, CH_3CO), 1.94 (s, 3 H, CH_3CO), 1.89 (s, 3 H, CH_3CO) and 1.69–1.57 (m, 4 H, noradamantane); ^{13}C NMR (100 MHz; $CDCl_3$) 170.42, 170.26, 169.89 (3 × O*C*OCH$_3$), 154.69, 154.47, 149.82 (C-2, C-4, C-6), 138.39 (C-8), 137.76, 137.63, 137.50 (3 × C-1 Bn ether ring), 128.77, 128.67, 128.54, 128.16, 128.04, 127.96, (Ar*C*H), 119.43 (C-5), 98.34 (C-1"), 86.22 (C-1'), 86.92, 74.84 (2 × CH), 73.95, 73.81, 73.55 (3 ×O*C*H$_2$Ar), 72.11 (CH), 69.63 (C-5' or C-6"), 69.52, 69.27 (2 × CH), 66.29 (C-5' or C-6"), 49.01 (CH$_2$, noradamantane), 43.57, 37.83 (CH, noradamantane), 35.32 (CH$_2$ noradamantane) 21.33, 21.14, 20.85 (3 × CH$_3$*C*O); MS: (FAB) *m/z* 980.3 [(M+H)$^+$, 80%], *m/z* calcd for

$C_{52}H_{58}N_5O_{12}Cl$ $[M+H]^{+\,37}Cl$ 982.3819 found *m/z* 982.3854, $[M+H]^{+\,35}Cl$ 980.3848 found *m/z* 980.3848:

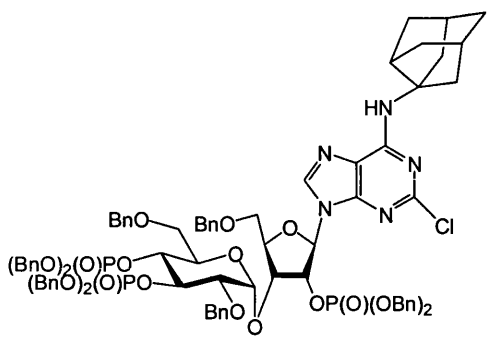
2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-*D*-glucopyranosyl-2-chloro-6-(3-noradamantylamino)-9-β-*D*-ribofuranosylpurine (100).



NaOMe (5 mg, 0.092 mmol) was added to a solution of **99** (90 mg, 0.092 mmol) in methanol (4 mL) and the mixture was stirred for 90 min. It was then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil, which was purified by flash chromatography on silica using dicloromethane–methanol (20:1) as eluent to give the title compound (65 mg, 84%) as a colourless oil.

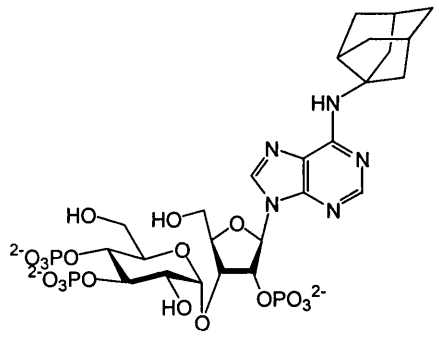
$[\alpha]^{20}_D$ +6.8 ± 1.0 (*c* 0.59, $CHCl_3$); ^1H NMR (400 MHz; $CDCl_3$) δ_H 7.98 (s, 1 H, H-8), 7.37–7.21 (m, 15 H, ArC*H*), 6.24 (d, 1 H, $J_{1',2'}$ 5.9 Hz, H-1'), 5.84 (br s, 1 H, N*H*), 4.69 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 4.63 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.59–4.36 (m, 7 H, H-2', H-3', 2.5 × OCH_2Ar), 4.15 (t , 1 H, $J_{3",2"}$ =$J_{3",4"}$ 9.8 Hz, H-3"), 4.06–4.01 (m, 2 H, H-4', H-5"), 3.95 (dd, 1 H, $J_{6"a,5"}$ 2.1 Hz, $J_{6"a,6"b}$ 10.5 Hz, H-6"a), 3.76 (t, 1 H, $J_{4",3"}$=$J_{4",5"}$ 9.4 Hz, H-4",) 3.66 (dd, 1 H, $J_{6"b,5"}$ 7.4 Hz, $J_{6"b,6"a}$ 10.5 Hz H-6"b), 3.51–3.44 (m, 2 H, H-5'a, H-5'b), 3.41 (dd, 1 H, $J_{2",1"}$ 3.5 Hz, $J_{2",3"}$ 9.8 Hz, H-2"), 2.64–2.61 (m, 1 H, noradamantane), 2.35–2.18 (m, 6 H, noradamantane), 2.02–1.94 (m, 2 H, noradamantane) and 1.69–1.56 (m, 4 H, noradamantane); ^{13}C NMR (100 MHz; $CDCl_3$) 154.70, 153.74, 149.61 (C-2, C-4, C-6), 138.34 (C-8), 138.16, 137.59, 136.71 (3 × C-1 Bn ether ring), 129.22, 128.96, 128.79, 128.47, 128.19, 127.96, 127.90, 127.77, 127.70 (Ar*C*H), 119.27 (C-5), 93.69 (C-1"), 87.97 (C-1'), 84.03, 81.59, 79.77, 77.68 (4 × CH), 73.92, 73.79 (3 ×OCH_2Ar), 72.82, 72.77 (2 × CH), 70.66, 70.41 (C-5', C-6"), 68.82 (CH), 48.92, 48.92 (CH_2 noradamantane), 43.58 (CH noradamantane), 43.58 (CH_2 noradamantane), 37.83 (CH noradamantane) and 35.38 (CH_2 noradamantane); MS: (FAB) *m/z* 854.2 [$(M+H)^+$, 52%], *m/z* calcd for $C_{46}H_{52}N_5O_9Cl$ $[M+H]^{+\,37}Cl$ 857.3535 found *m/z* 857.3583, $[M+H]^{+\,35}Cl$ 854.3531 found *m/z* 854.3502:

2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-*D*-glucopyranosyl- 2-chloro-6-(3-noradamantylamino)-9-β-*D*-ribofuranosylpurine (101).



Bis(benzyloxy)(diisopropylamino)phosphine (97 mg, 0.28 mmol) and 1*H*-tetrazole (29 mg, 0.42 mmol) were stirred together in dichloromethane (3 mL) for 30 min, the mixture thus obtained was then added to **100** (40 mg, 0.04 mmol). After a further 20 min, TLC (ethyl acetate:methanol, 95:5) indicated conversion of starting material to a single trisphosphite. The reaction mixture was then cooled to –78 °C and *m*CPBA (95 mg, 0.33 mmol) was added. After 10 min, 10 % aq. Na_2SO_3 solution (15 mL) and ethyl acetate (20 mL) were added and the mixture was allowed to heat up to room temperature. The resulting organic layer was washed with sat. aq. $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using ethyl acetate–hexane (2:3 then 1:1 then 4:1) to give the title compound (35 mg, 46%) as a colourless oil. ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.72 (s, 1 H, H-8), 7.51–6.99 (m, 45 H, ArC*H*), 6.24 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 6.06 (br s, 1 H, N*H*), 5.51–5.46 (m, 1 H, H-2'), 5.30 (d, 1 H, $J_{1",2"}$ 4.3 Hz, H-1"), 5.04–4.33 (m, 21 H, H-3', H-4', H-3", H-4" 8.5 × OCH_2Ar), 4.30 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 3.82–3.78 (m, 1 H, H-5"), 3.72–3.52 (m, 5 H, H-5'a, H-5'b, H-2", H-6"a, H-6"b), 2.63–2.59 (m, 1 H, noradamantane), 2.34–2.17 (m, 6 H, noradamantane), 2.09–1.98 (m, 2 H, noradamantane) and 1.65–1.56 (m, 4 H, noradamantane); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P –0.23, –0.91, –0.11; MS: (FAB) *m/z* calcd for $C_{88}H_{91}N_5O_{18}P_3Cl$ $[M+H]^+$ 1636.5309 found *m/z* 1636.5334.

3'-*O*-α-*D*-Glucopyranosyl-6-(3-noradamantylamino)-1-β-*D*-ribofuranosylpurine 2',3",4"-trisphosphate (102).



A mixture of **101** (10 mg, 0.0061 mmol) and $Pd(OH)_2$ on carbon (10%, 30 mg) in cyclohexene (3 mL), methanol (6 mL) and water (0.5 mL) was heated at 70 °C for 2 h. The catalyst was filtered and washed well with de-ionised water and methanol. The filtrate was concentrated under reduced pressure to a glassy solid. The residue was dissolved in de-ionised water (1 mL) and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was concentrated under reduced pressure to give the title trisphosphate (3.6 μmol, 59%) as the sodium salt.

^{1}H NMR (400 MHz; D_2O) δ_H 8.04 (s, 1 H, H-8), 8.03 (s, 1 H, H-2), 6.05 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1'), 5.19 (d, 1 H, $J_{1",2"}$ 3.5 Hz, H-1"), 5.06–5.02 (m, 1 H, H-2'), 4.57–4.22 (m, 3 H, H-3', H-4', H-3"), 3.83–3.55 (m, 7 H, H-5'a, H-5'b, H-2", H-4" H-5", H-6"a, H-6"b), 2.37–2.35 (m, 1 H, noradamantane), 2.18–2.01 (m, 6 H, noradamantane) 1.88–1.86 (m, 2 H, noradamantane) and 1.45–1.42 (m, 4 H, noradamantane); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.44, 2.49 and 2.49; MS: (FAB) *m/z* 788.0 [$(M–H)^-$, 100%], *m/z* calcd for $C_{25}H_{37}N_5O_{18}P_3$ $[M-H]^-$ 788.1301 found *m/z* 788.1336:

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